Emissions of Bioaerosols from Composting Facilities

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Abstract

Composting is a waste management procedure leading to the degradation of organic substances into stable end-products that can be used as fertilizer. However, one of the major public health concerns is the emissions of bioaerosols from the composting sites. Bioaerosol dispersion across composting facilities, due to operational activities, may have significant effects on human health due to the fact that they can penetrate into the human respiratory system leading to various toxic, allergenic and infectious diseases. Therefore, in this study emissions of bioaerosols from composting sites were characterized utilizing molecular techniques in order to investigate the microbial diversity and the extent of dispersal across facilities. Bioaerosols emissions were evaluated during different seasons at a UK composting site and for the warm season at two sites in Lahore (Pakistan) to assess the impact of seasonal meteorological factors on the composition and abundance of bioaerosols. Maximum abundance was found onsite with the minimum upwind. Maximum abundances of total bacteria (5.52×10^{10} copies/m^3) and *Saccharopolyspora rectivirgula* (8.7×10^8 copies/m^3) obtained during summer suggested a positive influence of temperature on microbial abundances. Minimum abundance of bioaerosols was obtained during the winter season. Further, microbial community composition analysis indicated differences in the bacterial and fungal populations between the compost and bioaerosols obtained from different locations of the composting sites. Emissions of bioaerosols were also investigated in laboratory scale composting experiments at different temperatures and moisture levels to gain further insights into the impact of atmospheric factors on the fate of bioaerosols. Higher temperatures led to the increased abundances of total bacteria and *Aspergillus fumigatus* but had a negative effect on the abundance of *Saccharopolyspora rectivirgula*. The findings of the study have indicated that seasonal variations along with other parameters impact on the dispersion and diversity of bioaerosols which is an important consideration in the re-evaluation of the risk assessment policy regarding composting facilities.

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Table of Contents

List of Abbreviations

AFOR – Association for organics recycling BMW – Biodegradable municipal waste

C/N – Carbon/Nitrogen

CFU – Colony forming unit CH_4 – Methane

CIWM – Chartered institution of waste management

cm – centimetre

CO_2 – Carbon dioxide

Defra – Department for environment, food & rural affairs

DGGE – Density gradient gel electrophoresis

DNA – Deoxyribonucleic acid

EDTA – Ethylenediaminetetraacetic acid g – gram

HCl – Hydrochloric acid

HSD – Honest significant difference

ITS – Internal transcribed spacer l – litre

M – molar

m/s – metre per second

m^2 – square metre

ml – millilitre

MLI – Multi-stage liquid impinge –

mm – millimetre

mM – millimolar

MOI – Multi-orifice impinger

MW – Molecular weight

NaCl – Sodium chloride

NS – Non significant

ng – nanogram

nm – nanometre

PCR – Polymerase chain reaction

pH – Potential hydrogen

QPCR – Quantitative polymerase chain reaction

RNA – Ribonucleic acid

RPM – Revolution per minute

SDS – Sodium dodecyl sulfate

TAE – Tris-acetate-EDTA

µl – microlitre

µm – micrometre

µM – micromolar

UV – Ultraviolet

List of Figures

List of Tables

APPENDIX

Chapter 1 Introduction

1.0 Background

In the UK, composting is a key component in the waste recycling strategy to reduce the quantity of waste going to landfill. Around 400 million tonnes of waste was generated in 2012 and that includes construction, commercial & industrial and household waste; out of which 7.54 million tonnes of organic material was processed through composting and anaerobic digestion (Horne *et al.*, 2013). According to the European Landfill Directive, there is an obligation to reduce the quantities of biodegradable municipal waste (BMW) sent to landfill to 35% of 1995 levels by 2020 (Wheeler *et al*., 2001; Swan *et al.*, 2003; Stag *et al.,* 2010; Pearson *et al.,* 2015). Further, the UK waste statistical notice (2016) of Defra stated that in 2014, 8.7 million tonnes of BMW was sent to landfill representing a decline of 24% from the 1995 target baseline.

Consequently, there has been an increase in the number of waste composting sites as well as increased capacity in existing ones. According to a survey of the UK organics recycling industry (2012), almost 323 composting sites were functional in 2012 with an overall composting capacity of 7.48 million tonnes (78%). About 5.85 million tonnes organic waste was sent to these facilities comprising of garden/green waste (63%), food waste (31%) and 6% others i.e. sewage sludge, manure, liquids and woods (Horne *et al.*, 2013).

Composting is a waste management procedure leading to the degradation of organic substances into stable end-products that can be used as fertilizer (Skyes *et al*. 2007). The core aim of the composting process is to avert the biodegradable waste going to landfill and hence reducing methane emissions, at the same time as recovering valuable resources. The degradation of organic matter is a dynamic process, governed by a progression of microbes with each group attaining its ultimate activity under optimum conditions (Fig 1.1). Effective microbial decomposition of organic matter depends upon controlled conditions of temperature, moisture content and pH, resulting in rise in a temperature due to microbial activity (Wéry, 2014).

Composting is generally described as the microbial decomposition of organic matter under aerobic environment. However, occasionally it undergoes anaerobic decomposition leading to a

process called fermentation (Fig 1.1). In these processes, bacteria, fungi and moulds utilize waste organic matter to convert it into a more stable form. Based on their degradation activity relative to temperature of the compost these microorganisms are considered as mesophiles and thermophiles. Also, in order to understand the degradation mechanism in the composting process, mesophiles and thermophiles can also be regarded as first order consumers (feed directly on substrate); and second order consumers (utilize products generated by first order consumers). Usually, organic waste material undergoes processing at the waste facility before the composting process. Received wastes are shredded into smaller sizes to decrease the particle size and increase the surface area; adjustment of carbon and nitrogen ratio (C: N) ratio through addition and mixing of different feedstock materials; and screening of shredded waste to remove containments (Lane, 2003).

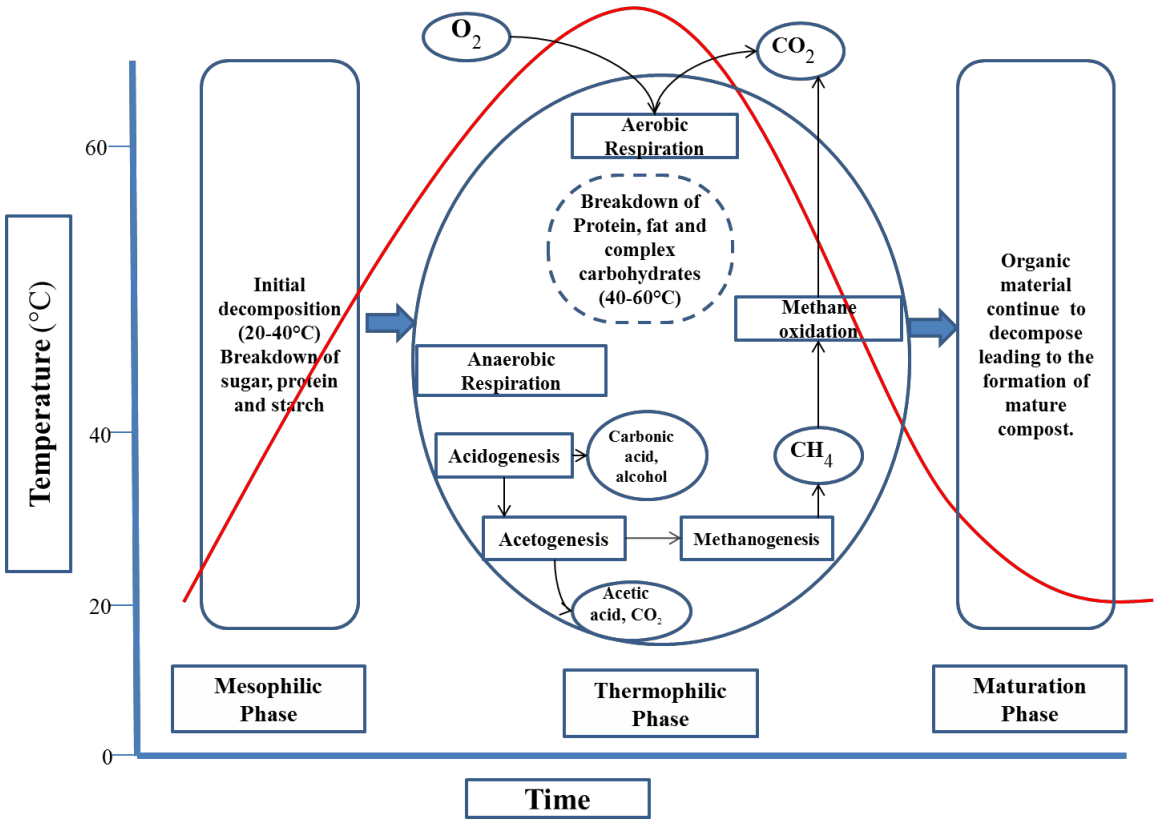

Figure 1.1 Phases of composting and associated processe

1.1 Phases of composting

Depending on temperature, composting has been defined in three key stages, i.e. mesophilic phase, thermophilic phase and curing/maturation phase (Fogarty & Tuovinen, 1991; Miller, 1996), as shown in figure 1.1.

1.1.1 Mesophilic phase (initial stage)

Mesophilic microbes grow at moderate temperatures typically ranging from 20°C to 40°C. Mesophilic microbes such as, bacteria (*Streptococcus* spp.), fungi (*Cladosporium* spp) and actinomycetes (*Streptomyces* spp.) start microbial activity through the breakdown of organic matter, resulting in a rise in temperature from 10°C to 40°C. Normally, it takes almost 2-4 days to attain the mesophilic optimum temperature range from ambient temperature depending upon the environmental settings. Microbial decomposition of organic material is dynamic in nature involving a succession of microorganisms i.e. bacteria, fungi and actinomycetes, whereby each group (bacteria & fungi) attaining its peak activity under optimum conditions. As a result of microbial activity, simple carbohydrates and proteins are decomposed during this stage of the composting process.

1.1.2 Thermophilic phase

Thermophilic microbes thrive at high temperature ranging from 40°C to 80°C. Thermophilic microbes take over the process due to successive microbial activity and consequent increase in temperature above 45°C. Typically described as the active phase of the composting process, it involves a gradual rise in the temperature of the compost pile to 45-60°C within 24-72 hours and maintained there for weeks. During the same period, oxygen is supplied through aeration approaches i.e. turning of the piles and forced aeration, to maintain microbial activity, thus utilizing the aerobic respiration process for the breakdown of complex carbohydrates, proteins and fats (Chen *et al*., 2011; Debertoldi *et al*., 1983).

However, during anaerobic conditions microbial breakdown of organic matter results into the production of methane and carbon dioxide via a series of processes i.e. acidogenesis, acetogeneis and methanogenesis. Acidogenesis is an acid- forming stage where products of mesophilic decomposition are fermented into carbonic acids (acetic acid, butyric acid), ethanol and CO_2. Conversion of glucose to ethanol and propionate are main reactions involved in acidogenesis (Ostrem, 2004). Further the transformation of the products of acidogenesis can result in acetate, CO_2 and H_2. The chemical reactions comprising this stage involve conversion of glucose, ethanol, propionate, and bicarbonate into acetate (Arsova, 2010). Finally, the conversion of acetate, alcohol and CO_2, produced in the former stages of anaerobic respiration, into methane (CH_4) is known as methanogenesis which is facilitated by methanogenic microorganisms (Verma, 2002).Thermophiles usually function between 45°C and 70°C and are involved in the degradation of highly complex carbohydrates, proteins and amino acids. Moreover, the high temperature during the composting process is attributed as the pasteurization of pathogenic microbes, accelerating the microbial activity and rate of decomposition of organic matter (Nakasaki *et al.*, 1985).

1.1.3 Maturation phase

This is characterized by a decrease in compost temperature to ambient because of subsiding microbial activity. However, during this phase biological materials continue to decompose due to recolonization of mesophilic actinomycetes and fungi into eco- sustainable humic material-mature compost which is then utilized as organic fertilizer. Normally, the time period for the maturation phase ranges between 1 to 4 months, nevertheless, it can be increased if the compost is immature or unstable so that it contains a high amount of organic acids, extreme pH values and high salt concentrations.

1.2 Factors influencing the composting process

Several biotic and abiotic factors are primarily important in composting to obtain a stable and sustainable end product. As a microbe-driven process, controlled and optimum parameters are

necessary for natural decomposition and attaining a high quality yield. The interactions and effect of these factors in the composting environment also provides an understanding of variations in microbial community composition and abundance.

1.2.1 Temperature

As the microbes degrade organic substances heat is released and the temperature of the compost rises gradually (self-heating). Apart from self-heating of compost matter due to microbial activity various studies have assessed the effect of different external temperatures on the composting process (Macgregor *et al.*, 1981; Strom, 1985; Mosher & Anderson, 1977; Miller *et al*., 1989; Tiquia *et al.,* 1996).

Temperature affects the microbial metabolism as well as abundance and composition of microbes during the composting process (Liang *et al.*, 2003). In order to evaluate the relationship between microbial activity and temperature during the composting process McKinley and Vestal (1984) stated that degradation of organic matter can be achieved efficiently between 25°C to 45°C as compared to higher temperatures i.e. 55°C to 74°C. Furthermore justification to effective microbial activity at low temperatures can be provided from a laboratory scale bio-solid composting experiment at controlled temperatures and moisture content settings (Liang *et al.*, 2003) which indicated that higher microbial activity can be accomplished at low temperatures (22°C - 36°C) and high moisture content (60% - 70%).

However, low temperatures i.e. below 20 °C can inhibit the composting process (Table 1.1) (Mosher & Anderson, 1977) whereas quite higher temperatures i.e. 82 °C impede the microbial degradation of organic matter (Miller *et al*., 1992; Finstein *et al*., 1986). On the basis of maximum CO_2 evolution due to microbial activity (Suler & Finstein, 1977; Nakasaki *et al.*, 1985) it has been suggested that 60°C is the optimum temperature for composting. Also, Strom (1985) found a decrease in thermophilic bacterial diversity when composting functioned above 60 °C. These findings suggested that the optimum temperature for composting ranges between 55-60 °C.

Table 1.1 Effect of temperature and moisture content regimes on composting process

Temperature	Moisture content	Composting process
<20°C	<40%	slow or even stop
52°C – 60°C (optimum)	50-60% (optimum)	maximum decomposition
>60°C	70%	microbial activity declines

1.2.2 Moisture content

Moisture content helps in the transportation of nutrients to composting microbes for their microbial activity (McCartney & Tingley, 1998). Low moisture content (<40%) affects the movement of microbes within the compost that lead to the desiccation and ultimately stopping the composting process (Table 1.1) (Debertoldi *et al*., 1983). Conversely, high levels (70%) of moisture cause anaerobic conditions as it limits transport of oxygen by filling small pores between particles (Tiquia *et al.,* 1996).

Richard *et al. (*2002) found that the optimum moisture level for microbial decomposition ranged between 50-70%. Other studies (Suler & Finstein, 1977; McKinley & Vestal, 1984) indicated that optimum moisture content ranged between 50-60%. Tiquia *et al*. (1996) showed that an increase in moisture levels up to 70% reduced the microbial activity in composting systems. This discrepancy in optimum moisture level may be due to the variations between different compost substrates and during different times of the composting process. Additionally, operating outside the optimum range of moisture content had a considerable adverse effect on the microbial activity and slows down or terminates the composting process (Richard *et al.*, 2002).

1.2.3 Carbon/ Nitrogen Ratio

Maintaining an optimum carbon/nitrogen ratio is necessary for the suitable microbiological decomposition as carbon is termed as an energy source for microbial action while nitrogen determines the growth of the microbial population. Usually in effective composting, microbes utilize 30 parts of carbon to 1 part of nitrogen however if ratio surpasses 35 it would decrease

the microbial activity. Furthermore a decrease in ratio below 25 causes conversion of nitrogen into ammonia producing odours into the atmosphere (Pace *et al.,* 1995). Nitrogen is a vital component and a part of amino acids and nucleic acids which play a significant role in maintaining biological processes in all forms of life. Nitrogen is utilized by microbes to form proteins which are essential in cell growth and repair. Nitrogen limited conditions affect the synthesis of proteins, amino acids, DNA, and RNA which hinders the growth and repair mechanisms of microbes. Consequently, compost undergoes cooling phase as a result slow degradation of organic matter depending on the availability of nitrogen. Nitrogen fixing bacteria obtain nitrogen directly from atmosphere and convert it into ammonia which can be combined with carbon to synthesize amino acids (Todar, 2013). Similarly, Phosphorus is utilized for energy transfer in the cells in the form of Adenosine triphosphate (ATP) which is an essential component in nucleic acids and phospholipids (cell membrane). Deficiency of phosphorus limits the growth of microorganisms (Talaro, 2006).

Generally, a C/N ratio of 25 to 30 is considered as the optimal fraction for composting (Fong *et al*., 1999). Moreover, in a pilot scale study Zhu *et al.* (2004) composted swine manure with rice straw utilizing C/N ratio of 25 in natural, passive and forced aeration schemes and suggested passive and forced aeration to be employed for small and large scale composting facilities respectively. However, some studies suggested that efficient composting can be achieved at a low C/N ratio. Zhu (2007) concluded a C/N ratio of 20 was viable whilst examining the effect of initial C/N ratio in a pilot scale study on the composting of swine manure with rice straw. Further Kumar *et al.* (2010) demonstrated that the effective decomposition of green waste can be achieved at a C/N ratio of 19.6.

1.2.4 Feedstock composition

Feedstocks are the raw materials that undergo decomposition due the action of microorganisms resulting into a stable product. Different kinds of animal and agricultural waste can be utilized in the process of composting such as, cow manure, swine manure, chicken manure, crop remains, sawdust and rice straw (Kadir *et al.,* 2016). It is vital to blend carbon rich source (dried leaves, twigs, straw) with nitrogen rich source (fruits, vegetables, manure) in order to balance

nutrients requirement for the action of microbes and achieve efficient degradation of organic matter.

Diversity in feedstock composition results in physical, chemical and biological variations during the composting process as well as in the final end product (Cook *et al.,* 1994; Adams & Frostick, 2009). It has also been suggested that feedstock composition of organic matter affects the microbial community composition of the composting. Therefore, difference of feedstock influences the microbial composition and abundance of the bioaerosols. Epstein, (1994) reported that different composition of waste material results in the diverse microbial communities during the composting process. Similarly, Swan *et al.* (2003) indicated variations in microbial composition and abundance on the basis of different types of waste employed in the composting services.

1.2.5 Microbial communities

Different types of microbial communities, dominate the different phases of composting (Debertoldi *et al.*, 1983) (Table 1.2).The degradation capacity of microbes primarily depends upon their ability to produce enzymes essential for decomposition of organic components (Tuomela *et al.*, 2000). Initial degradation comes from endophytes and microbes present in the raw material or in the air, resulting in a gradual rise in temperature, eventually followed by thermophilic bacteria, fungi and yeast. This shift in temperature also affects the species diversity, for instance *Bacillus* species dominate the thermophilic phase of composting (Strom, 1985). Other studies also showed that *Firmicutes* and *Actinobacteria* dominate during thermophilic phase of composting (Le Goff *et al.*, 2010; Tang et al., 2007). Apart from the *Bacillus* spp., the dominant bacterial thermophiles usually include *Thermoactinomyces, Geobacillus, Planifilum, Saccharomonospora, Thermobifida* whereas; *Thermomyces, Aspergillus* and *Penicillium* are the dominant fungal species thermophilic aerosols (Wery, 2014).

Simple and soluble sugars and organic acids are easily digested by heterotrophic and heterogeneous microbial species. It has been found that several species of eumycetes,

mesophilic and thermophilic microbes are responsible for the decomposition of starch, pectin cellulose and lignin. However, the decomposition of cellulose is a quite complex and intensive process and degraded by fungi belonging to Ascomycota, Basidiomycota and Deuteromycota genera.

In contrast to cellulolytic fungi, the number of cellulolytic bacteria decreases during the later stages of composting (de Bertoldi *et al.*, 1983). The degradation of lignin, an internal cell wall component of plants, is largely carried out by species belonging to Basidiomycotina and partially by Ascomycotina genera (Tuomela *et al.*, 2000) (Table 1.2). Also, many genera of actinomycetes have the ability to degrade and solubilize the structure of lignin. Similarly, cellulose and hemicellulose biodegradation into soluble disaccharides and glucose is achieved through cellulolytic enzymes i.e. endo & exo-glucanases and glucosidases by cellulose degrading anaerobic bacteria, for example, *Clostridium cellobioporus, Clostridium thermocellum, Clostridium lochhadii* and *Bacterioides succinogenes* (Barlaz & Palmisano, 1996). During anaerobic decomposition of carbohydrates, lipids and proteins, the production of alcohols and CO_2 is attained by the action of facultative bacteria, for example, *Enterobacter* spp. and *Streptococcus* spp. It has been indicated that several anaerobic species of bacteria are involved in the degradation of proteins into amino acids, peptides and carbon dioxide including *Clostridium perfringens, C. sporogenes C. histolyticum* and *C. bifermentans* (Palmisano & Barlaz, 1996). The monomers produced from the complex substances are transformed to lactate, propionate, succinate, and alcohols by number of fermenters and acetogens mainly including i.e. *Syntrophobacter wolinii, Clostridium* spp, *Actinomyces, Lactobacillus Thermacetogenium phaetum, Pelotomaculum thermopropionicum* and *Thermosintrophicum* (Verma, 2002; Arsova, 2010). The production of methane is accomplished through acetate-oxidising bacteria and methanogenic archaea (also known as methane formers). The breakdown of acetic acid molecules into methane and CO_2 is carried out by number of methanogens including *Methanobacillus, Methanobacterium, Methanosarcina and Methanococcus* and *Methanobrevibacter*. However, *Methanosaeta and Methanosarcina* species have been

suggested as dominant methanogens in mesophilic and thermophilic stages of large scale sewage sludge and water treatment plant due to their tolerance for wide range of temperatures and nutrients (Palmisano & Barlaz, 1996).

Table1.2 Dominant microbial flora present during composting (Debertoldi *et al.*, 1983; Tuomela *et al.*, 2000; Partanen *et al.*, 2010)

Bacteria	**Fungi**
Bacillus coagulans	*Aspergillus fumigatus*
Bacillus circulans	*Coprinus* sp.
Bacillus subtilis	*Penicillium* sp.
Bacillus licheniformis	*Scytalidium thermophilim*
Bacillus megaterium	*Termonmyces* sp.
Bacillus pumilus,	*Tricoderma* sp.
Bacillus stearothermophilus	*Papulaspora thermophilia*
Clostridium thermocelium	*Myriococcum thermophilium*
Escherichia coli	*Malbranchea pulchella*
Streptocuccus	*Chaetomium thermophilum*
Pseudomonas sp.	*Ganoderma colossum*
Thermoactinomyces sacchari	*Phanerochaete*
chrysosporium Thermoactinomyces vulgaris	
Thermomonospora	
curvata	
Thermoactinomyces	
viridis Nocardia sp.	
Micorpolyspora faeni	
Actinobifida ahromogena	

1.3 Bioaerosols

Aerosols are liquid droplets or solid particles that are suspended a gas and range in size from 0.001 to 100 µm. Bioaerosols are airborne particles that have a biological source and include microorganisms (bacteria, fungi, viruses) and biological compounds such as toxins and debris from membranes (Sykes et al. 2011). Generally, bioaerosols include pathogenic or non-pathogenic, viable or non-viable mi crobes and their cellular waste by- products such as endotoxin, mycotoxin, β(1-3) glucan and plant fragments (Douwes et al.2003). Due to their ubiquitous nature bioaerosols are found in both the indoor and ambient environment (Reponen, 2011). There are different sizes of the constituents of bioaerosols. The size of Viruses ranges from 15-400 nm; whereas, bacterial and fungal size ranges from 0.3-10 µm and 1-100 µm respectively (Colbeck & Nasir, 2010; Macher et al. 1998). Bioaerosols can be produced by any activity that results in microbial contaminated material becoming airborne. During handling and disruption of compost material, microorganisms and their cellular by-products may become airborne. Various operational activities (e.g. shredding and screening) at composting sites increase the concentration of microbes in the air (Persoons et al. 2010). Bioaerosol dispersion during the composting process may have significant effects on human health due to the fact that they can penetrate into the human respiratory system leading to various toxic, allergenic and infectious diseases (Wheeler et al., 2001; Swan et al., 2003; Walser et al., 2015).

On the basis of the research conducted by Wheeler et al. (2001), the Environment Agency has concluded that bioaerosol concentrations are reduced to background levels within 250 m of open composting facilities. From 2001, the Environment Agency has been giving permits to those operators who have provided site specific risk assessment if there is dwelling or workplace within 250 meters of compost sites to keep the concentration of bioaerosols at acceptable levels as follows 1000 CFU/m^3, 500 CFU/m^3 and 300 CFU/m^3 respectively for total bacteria, Aspergillus fumigatus and Gram negative bacteria (Wheeler et al., 2001;

AfOR, 2009; Defra, 2013). Douglas et al. (2016) concluded that, for the UK, there is no evidence of increased risks in respiratory hospital admissions for residents living beyond 250 m of composting sites. A number of studies have been conducted to investigate the emission of bioaerosols or their derivatives from composting facilities (Wéry, 2014 and references therein; Defra, 2013; Health and Safety Executive, 2010). Additionally, an evaluation of the quality of risk assessment, reviews of various methods and protocols for the monitoring of bioaerosols from compositing facilities have also been produced (Drew et al., 2009; Environment Agency, 2009; Association for Organics Recycling, 2009).

Exposure to bioaerosols from composting facilities can cause serious effects on workers respiratory health i.e. organic dust toxic syndrome, extrinsic allergic alveolitis, asthma, mucous membrane irritation etc. (Skyes et al., 2007). In occupational health based studies, symptoms related to respiratory system, skin inflammation, watering eyes, eye irritation, itching eyes, sore throat, watering and blockage of nose and conjunctivitis were reported among compost workers (Bunger et al., 2000, 2007; Hambach et al., 2012; Van kampen et al., 2012).

Furthermore some residential related health studies found no significant symptoms of respiratory system and association between A. fumigatus and asthma, allergy, eye, nose and throat irritation, joint pain, nausea, cold and flu (Browne et al., 2001). However in Herr et al., (2003), residents living within 150-200m of composting facility showed symptoms of respiratory system, eyes irritation and excessive tiredness. While reviewing the published literature to determine the exposure limits of the hazardous effects of bioaerosols, Walser et al. (2015) reported scarcity of exposure- response relationships due to deficiency of dose – response information, utilization of different bioaerosols sampling and analysis methodologies, diversity in health issues and limited knowledge about exposure assessment.

According to Control of Substances Hazardous to Health Regulations (2013), bioaerosols that are emitted from composting facilities are hazardous to health. Exposure to bioaerosols is by inhalation mainly during handling of the compost material. The risk of ingestion can be minimized by the following hygiene promoting practices: wearing of gloves, mouth mask

and hand washing. However, mitigation of bioaerosol emissions from composting facilities is a complex issue and requires knowledge of the microbial signature produced during on-site activities i.e. shredding, screening and turning.

1.3.1 Bacteria

In general, gram negative bacteria are abundant in dusts of plant origin and include species such as *Pseudomonas spp., Klebsiella spp., Pantoea agglomerans, Rahnella spp.* While gram positive bacteria mainly predominate in dusts of animal origin, some species such as, *Bacillus spp, Corynebacteria, Staphylococcus spp., Micrococcus spp*., and *Streptococcus spp*. are also found in plant dusts (Dutkiewicz, 1997). Consequently, these bacterial species are more likely to be present in the composting of green waste.

Actinomycetes, filamentous gram positive bacteria, are different from other bacteria due to their role in decomposition of the organic matter and are commonly present in moist and aerobic settings of soil and plant habitats (Goodfellow & Williams, 1983). During the process of self-heating in composting, actinomycestes can easily be observed through long greyish threads resembling spider webs and the soil- like odour of compost material. Due to these characteristics, they can be used as an indicator of the process of self-heating and as well as an indicator organism showing the presence of bioaerosols emitted from compost (Lacey & Crook, 1988). During the handling of compost, actinommycetes spores are produced and aerosolized in large quantities. The airborne spores of thermophilic actinomycetes (*Thermoactinomyces vulgaris, Saccharopolyspora rectivirgula)* are known to cause respiratory illness i.e. hypersensitivity-induced pneumonitis and other allergic reactions such as alveolitis or bronchial asthma. Primarily, these are responsible for lung diseases such as Farmers Lung Disease and Mushroom Workers Lung Disease (Lacey & Crook, 1988; Swan *et al.*, 2003; Wéry, 2014). Common species of actinomycetes found in compost aerosols are *Saccharomonospora viridis, Saccharopolyspora rectivirgula, Thermoactinomyces thalpophilus, Thermoactinomyces vulgaris, Thermomonospora spp, Streptomyces* spp. (Goodfellow & Williams, 1983; Swan *et al.*, 2003). Further, pathogenic Gram negative bacteria of animal faecal origin such as *Salmonella spp, E. coli* and

Campylobacter can be found in composting of animal manures and sewage sludge (Swan *et al.,* 2003). Gram negative bacteria dominate the mesophilic phase of composting process however heating of compost leads to the thermophilic phase which is dominated by gram positive bacteria. The production of high temperatures ($50^{O}C$ – $70^{O}C$) during composting kills most of the pathogens, yet some pathogenic species i.e. *Salmonella* spp, *E. coli* and *Bacillus spp*. can survive intense heat due to mishandling of compost piles (inadequate turning). Generally, the presence of bacteria in compost and in aerosols emitted from that compost depends on the nature and origin of waste and storage and handling conditions (Swan *et al.*, 2003).

1.3.2 Fungi

Depending upon their ability to survive at specific temperature, dynamics in fungal species are observed during different phases of composting. However, *A. fumigatus* and *Penicillin* are commonly found during composting (Drew *et al.,* 2009).

Saprophytic fungi are abundantly present during the handling of green waste and include *Alternaria* spp*, Cladosporium* spp and *Verticillium.* The proliferation of fungi through sporulation leads to the production of spores that are usually found in ambient air in low numbers. These fungi grow in stored organic material and common species are *Penicillium, Trichoderma, Aspergillus, Eurotium, Mucor* and *Rhizopus.* Inhalation of fungal spores leads to severe respiratory illness and workers in different working settings can be exposed to fungal spores thus inducing allergies, asthma and respiratory reponses (Swan *et al.*, 2003). Due to generation of large number of spores, *Aspergillus fumigatus* is widely distributed and ubiquitous ranging from 1 – 100 µm spores in indoor and outdoor environments. However, inhalations of spores are reported to have rare adverse consequences because of removal from immune system (Latge, 2001). It has also been suggested that several hundred spores of *A. fumigatus* are inhaled by humans per day without any severe outcome (Latge, 1999).

1.3.3 Endotoxins

Endotoxins are toxic substances, which are comprised of lipopolysaccharides and form the

outer membrane of gram negative bacteria. The “lipid A” portion of lipopolysaccharides enhances the toxicity of endotoxins (Searl & Crawford, 2012). While growing, minute quantities of endotoxins are produced and released. They are released in large amounts after cell disintegration.

Exposure to endotoxins can cause organic dust toxic syndrome (ODTS) which includes flu-like symptoms, fever, myalgia, and malaise; however chronic exposure to endotoxins is responsible for chronic bronchitis, chronic obstructive pulmonary disease and reduced lung function (Vogelzang *et al.*, 1998; Rylander, 2002). Studies have reported a relationship between the quantity of endotoxins in different environments and respiratory symptoms and suggested guidelines for environmental exposure to endotoxins (Thorn, 2001; Rylander, 2002; Wéry, 2014). Endotoxin is found in the cell wall of gram-negative bacteria and prolonged exposure to it can cause chronic bronchitis, chronic obstructive pulmonary and reduced lung function (Jacobs *et al.* 1997).

Regardless of the beneficial product (fertilizer) of composting, there are numerous health concerns associated with the occupational and community exposure to bioaerosols from composting sites. Bioaerosol exposure can have a significant impact on human health and usually presents as signs of inflammatory diseases, cough and fever (Enivors, 2004). Due to the size of bioaerosols they are unable to filter out by nasal hairs during inhalation and so penetrate into the lungs and cause respiratory and gastrointestinal diseases (CIWM, 2002). Invasive aspergillosis, severe infection in immuno-suppressed individuals, is caused by an opportunistic pathogen *Aspergillus fumigatus* (Taha *et al*. 2006; Allmers *et al.* 2000).

1.3.4 Mycotoxin

Mycotoxins are low molecular weight toxic secondary metabolites, produced by filamentous fungi. The term “Mycotoxicosis”, is generally understood to refer to the toxic effect of mycotoxins on humans and animals; however it may become severe due to a number of factors such as, toxicity of mycotoxins and their level of exposure, age and nutritional status of individuals and synergistic effects of other chemicals (Peraica *et al.*, 1999). The

significant sources of mycotoxicosis are consumption of contaminated food, skin contact with mould surfaces and inhalation of toxins that can cause a range of neurotoxic, carcinogenic and teratogenic effects in vertebrate's animals. Organic dust generated during the handling of compost material contains *A. fumigatus* and *Penicillium* spp which are responsible for the production of mycotoxins. Despite its toxicological effects, researchers have not studied the exposure to mycotoxin from compositing sites in detail (Swan *et al.*, 2003).

Whilst accessing the presence of secondary metabolites of *A. fumigatus* utilizing a culturing technique, (Fisher *et al.,* 1999) reported mycotoxins production by *A. fumigatus* in extracts of total dust and bioaerosols emitted from a composting facility. However, production of highly toxic mycotoxins associated with *A. fumigatus* (gliotoxin and verruculogen) was not found in the bioaerosols. Conversely, in another study Fischer *et al.,* (1998) reported no production of mycotoxins in organic dust and aerosols from composting facilities. Further, previously published literature has not indicated occupational health risks associated with mycotoxin; however their toxicity warrants further research (Swan *et al.,* 2003).

1.3.5 Glucan

(1-3) β-D-glucan are polysaccharides naturally found in the cell wall of fungi, bacteria, plants, and commonly found in the dust generated from waste. Exposure to β- Glucan during bioaerosol inhalation may result in the development of inflammatory responses resulting in adverse respiratory and lungs symptoms (Swan *et al.,* 2003; Searl & Crawford, 2012).

A considerable amount of literature has been published on the association of exposure to bioaerosols and airway inflammation, lung function, occurrence of atopy in indoor and occupational settings (Douwes *et al.*, 2000; Thorn & Rylander, 1998). In a study accessing the seasonal exposure to bioaerosol components among household waste collectors, Thorn (2001) reported a higher amount of glucan in the warm season and suggested a relationship between exposure to glucan and ambient temperature. (1-3)β-D-glucan is found in the cell wall of fungi, some bacteria and plants and exposure to it can increase inflammation

(Douwes *et al*., 2000). Inhalation of organic dust generated during composting processes can induce immunological respiratory signs and can cause allergic rhinitis, allergic asthma, chronic bronchitis, extrinsic allergic alveolitis and toxic pneumonitis (Chan-Yeung *et al*., 1992; Lacey, 1990; Lacey and Crook, 1988; Rylander, 1994).

1.3.6 Microbial signature of bioaerosols from composting facilities

Compost is an extraordinary environment with high and dynamic microbial abundance and composition during different phases of composting. During handling of compost material (shredding, turning and screening), the emission of bioaerosols is inevitable and there are significant concerns about the resultant exposure to workers as well as residents living in close proximity to such facilities.For more than a decade, the microbial diversity of bioaerosols from composting has been characterized through traditional culture based tools. As such studies mainly focused on cultivable bacteria, *actinomycetes* and *Aspergillus fumigatus* and these were considering as the dominant composting microbes. (Hryhorczuk *et al*., 2001; Kampfer *et al*., 2002). Culture based analysis leads to an underestimation of the true nature of the microbial signature of the bioaerosols from composting sites. Albrecht *et al*. (2007) highlighted the low enumeration (only 1.5-15.3%) of airborne bacterial from composting sites by direct counting after incubation on TSA Agar. This discrepancy is mainly due to the specificity in growth conditions of the medium for cultivable microbes. However, recent molecular advances of high throughput sequencing have greatly revealed the microbial diversity (Pankhurst *et al.*, 2012; De Gannes *et al.*, 2013). Moreover, in recent years a few studies have been conducted for the assessment of bioaerosols from composting sites through molecular tools.

Modern studies utilising 16S and 18S rRNA genes have significantly enhanced the in-depth understanding and characterization of the microbial signature of the bioaerosols emitted from composting facilities. The most common molecular method undertakes amplification and sequencing of genes encoding small subunit ribosomal RNA from environmental sources (Tringe & Hugenholtz, 2008). This approach has changed our understanding of microbial composition and abundance by discovering novel microbes that are uncultivable

in growth medium (Hugenholtz *et al.*, 1998). The use of high throughput next generation sequencing plays a significant role in investigating the unexplored microbial diversity and their interaction with the environment (Kelley & Gilbert, 2013).

In a study focusing on the bacterial and fungal community signature Bru-Adan *et al.* (2009) indicated a high diversity of bioaerosols emitted during the screening of mature compost. Comparison of phyla distribution of bioaerosols sample with compost and air sequences existing in the public database showed *Firmicutes* and *Actinobacteria* were the dominant bacterial phylotypes with 46% and 28% sequences respectively. *Bacteriocedetes* were dominant in compost and air sequences than bioaerosol sample; whereas *Actinobacteria* was found abundant in bioaerosols emitted from screened compost. The percentage of *Alphaproteobacteria* in screened-compost aerosols sample remained similar when compared with air sequences (Bru-Adan *et al*., 2009).

Furthermore, similar findings regarding microbial community structure were obtained when accessing the microbial flora of the thermophilic phase of the composting process (Le Goff *et al.*, 2010). However, themophlic bacterial and fungal phylotypes were abundant as compared with mature screened compost and that indicates intense microbial activity during the thermophilic phase. From the total bacterial phylotypes identified, 49.1% belonged to *Firmicutes* and 37.2% phylotypes to *Actinobacteria*. The dominant bacteria for *Fermicutes* were *Bacillus spp*., *Thermoactinomyces* and *Geobacillus* and for phylum *Actinobacteria, Saccharomonospora, Thermobifida* and *Saccharopolyspora*. Dominance of *Actinobacteria* is possibly due to their spore forming nature and spores from *Actinomycetes* can easily become airborne and disperse (Nielsen *et al.*, 1995). In assessing the concentration of microbes in bioaerosols of compost from household waste Nielsen *et al.* (1997) showed that *Actinomycetes* were prone to becoming airborne. Taha *et al.* (2005, 2007) described that the small size of *Actinomycetes* spores is a reason why these become airborne when compost is disturbed.

Pankhurst *et al.* (2012) investigated the effect of microbial community distribution in compost, upwind and more significantly downwind of the composting facilities and

phylogenetic analysis of bacterial communities revealed that *Firmicutes, Actinobacteria, Alpha* and *Gama proteobacteria* were dominant phyla in all samples whereas downwind samples lacked *Actinobacteria*. While the spore forming and airborne nature of *Actinobacteria* indicates their downwind dispersal in abundance they were abundant in upwind and in on-site samples (Le Goff *et al.*, 2010).

The results from Pankhurst *et al.* (2012) indicated daily variations in microbial community structure and distribution. Phylum *Proteobacteria* dominated compost, on-site and downwind (day 2) air samples, whereas upwind (day 3) samples were almost equally dominated by *Actinobacteria* (34%), *Proteobacteria* (32%) and *Firmicutes* (31%).On-site (day 3) samples were dominated by *Firmicutes* (75%). Furthermore, a high level of *Pseudomonas* was obtained in downwind air samples. These differences in the composition and abundance of microbial flora of bioaerosols primarily depends on various aspects i.e. meteorological conditions, activities at a specific area and time, mechanical and manual handling of the compost material and topography of the compost facility (Jones & Harrison, 2004). Pankhurst *et al.* (2012) showed daily variations in microbial diversity, nevertheless there is paucity of information to evaluate the causes of these variations. Moreover, this study also evaluated the downwind dispersal of the bioaerosols through molecular tools; however there is scarcity of information on the background concentration of bioaerosols. Thirty nine phylotypes were also obtained that shared homology with humans and plants phylotypes containing pathogenic species.

Fungal flora characterization during screening of mature compost was mainly dominated by *Basidiomycetes* (59% sequences) whereas among *Ascomycetes*, 9% of sequences represented *Aspergillus spp* and *oomycetes* group was represented by 12% of sequences (Bru-Adan *et al.*, 2009). Moreover during the thermophilic phase, *Ascomycota* was found as the dominant fungal phyla with 67% dominance and 19% of phylotypes were assigned to *Mucoromycotina*. *Aspergillus,*and *Thermomyces* and *Penicillium* were represented as the dominant fungi (Le Goff *et al.*, 2010). Whilst these phylotypes can originate from sources other than compost it is hard to determine the compost origin of these fungi. However

previous published literature has provided data about some of them in bioaerosols from composting. *Aspergillus fumigatus* is considered the most abundant fungi in aerosols emitted from composting sites (Albrecht *et al.*,2007; Bru-Adan *et al.*, 2009). Concerning bacterial phylotypes, only *Saccharopolyspora rectivirgula* was common in all five sites whereas common fungal phylotypes were identified as *Thermomyces lanuginosus* (Le Goff *et al.*, 2010).

Although there was a difference in level of diversity of bacteria and fungi at each site, the same bacterial and fungal phylum dominated each composting facility. *Ascomycota* dominated among fungi whereas *Firmicutes* and *Actinobacteria* dominated among bacteria.

In contrast to culture dependent techniques that underestimate the exposure to bioaerosols from composting facilities (Albrecht *et al.*, 2007), Q-PCR is considered as a beneficial molecular technique for the quantification of microbes in different environmental settings (Betelli *et al.*, 2013). Also different studies have showed QPCR to be particularly useful for species and genus specific quantitation in bioaerosols (Schäfer *et al.*, 2013).

Le Goff *et al.* (2011, 2012) classified specific microbial groups as signature candidates of composting bioaerosols and quantified each indicator through Q-PCR. Three phylotypes specific to the thermophilic phase were selected (i.e NA07, NC38 and EQ05) and affiliated respectively with genus *Saccharopolyspora, Thermoactinomycetaceae* and *Thermomyces lanuginosus*. Other researchers have also indicated the compost origin of these phylotypes. Their concentration in natural environments is low and various studies have suggested the use of thermophilic microorganisms to evaluate the concentration of microbes emitted from composting facilities (Kampfer *et al*., 2002; Fischer *et al*., 2008).

Schäfer *et al.* (2013) also assessed concentrations of *Saccharopolyspora rectivirgula* as a specific exposure marker at workplaces accompanying composting facilities utilising Q-PCR and showed that a Q-PCR approach is appropriate for bioaerosol quantification. Williams *et al.* (2013) determined the concentration of Aspergillus fumigatus spores in compost bioaerosols through Q-PCR suggesting it as potential method of monitoring spores

emitted from composting sites. Moreover, Betelli *et al.* (2013) demonstrated a standard method for the quantification of *Thermoactinomyces vulgaris* to assess workers exposure to bioaerosols from composting sites. Therefore, evaluation of microorganisms using specific species as signature candidates seems more significant for future prospective in order to characterize emissions and exposure to bioaerosols from composting sites (Schäfer *et al.*, 2013).

Currently, there is paucity of information on the diversity, abundance and dispersion of bioaerosols from composting facilities. Most of the existing information is based on fixed site monitoring and culture-based methods and may not truly reflect the actual exposure of workers and nearby residents. Use of molecular techniques can greatly contribute to developing tools to characterize bioaerosols emissions and to quantify the risk of human exposure from composting facilities.

1.4 Bioaerosol sampling methods

Precise and inclusive sampling of aerosols from composting sites is the basic for the comprehensive measurement of hazards and risks pose to human health. Currently, different sampling methods are employed to obtain detailed records of risks associated with waste management facilities (Stagg *et al.*, 2010). Yet, no sampling scheme has been approved as a standard technique, because of a number of dissimilarities associated with bioaerosol collection mechanisms i.e. collection efficiency of the media utilized, sampling time and equipment cost and field strength related issues, discrepancy in risk monitoring and exposure assessment from open- windrow based composting facilities as well as in-vessel composting systems.

The collection of aerosols from composting facilities can be carried out through a number of techniques such as impaction, impingement, filtration, cyclone scrubbing, electrostatic

precipitation and sedimentation. The main sampling methods for the collection of microorganisms from composting sites for culture-based and/or molecular analysis include impaction, impingement and filtration. The samplers utilized include Andersen impactors,

biosampler impingers and IOM inhalable samplers as shown in figure 1.2. Researchers are also employing analytical approaches for chemical fingerprinting of microbial volatile organic compounds (MVOCs) emissions from different environments (Garcia *et al.,* 2016).

Figure 1.2 (i) Biosampler impinger; (ii) Andersen impactor; (iii) IOM inhalable personal sampler (Haig *et al.*, 2016).

1.4.1 Impaction

Impaction is an air sampling technique that facilitates inertial forces in microorganisms' collection. Air is passed through the sampler and particles are removed by changing the direction of air. Particles having higher inertia cannot follow the air stream and are thus impacted on collection surface due to centrifugal influence; whereas particles with low inertia pass along with the air flow (Haig *et al.,* 2016). Direct impaction, a common sampling technique, involves collection of aerosols onto agar plates. There are a number of different samplers i.e. Andersen six-stage, two stage & single stage samplers, Marple eight-stage impactor and MAS-100 sampler (Jensen *et al.*, 1992; Li, 1999). These samplers differ from each other depending on the shape and size of the inlet, number of stages within the samplers and medium for collection i.e. solid (glass slide), semi-solid (agar plate) or filter (gelatine) (Macher & Hansson, 1987).

The AFOR (2009) standardised protocol recommends at least two (preferably four) properly sterilized single stage Andersen samplers (Fig1.2) with a flow rate of 28.3 l/min. Multi stage

samplers hold the ability to separate the collected particles on the basis of size fractionation.

The main benefit of utilizing this method involves minimal post sampling processing as the aerosols are collected, cultured and counted directly onto agar plates. There are also disadvantages such as, over-loading of the agar plates in highly contaminated air, dryness of agar during longer period of sampling and unsuitability for the sampling of non- cultured microbes.

1.4.2 Filtration

Filtration used filters as a collection surface for aerosol sampling. Air of desired flow rate is passed through a filter for a selected period of time; afterwards filters are stored for subsequent analysis. The type of filter utilized in bioaerosol sampling is dependent on the downstream analysis. Teflon and polycarbonate filters are commonly employed for extraction techniques, whereas carbonate and mixed cellulose are used for epifluorescence and direct microscopy approaches (Reponen, 2011). This technique can be applied to both culturing and non-culturing microbes; however, it requires longer post sampling laboratory analysis as compared to the impaction samplers.

Filtration based approaches are considered as efficient for the sampling of bioaerosols due to operational simplicity and inexpensive features; yet information on the particle size distribution cannot be gathered. Filtration may also be subject to overloading when operating in highly contaminated environments (Eduard & Heederik, 1998). The main problem associated with filtration is the desiccation of microbes when sampling for longer periods of time (>1h). It has been reported that fungi and spore forming bacteria can survive the desiccation stress whereas viability of vegetative bacterial cells decline during higher dryness (Wang *et al*., 2001).

1.4.3 Impingement

Impingement is similar in operation to that of impaction, but it involves collection of bioaerosols into a liquid medium. A solution of phosphate buffer and peptone water is often used as the collection medium while non-water based liquid medium is also employed i.e.

glycerol and mineral oil (Reponen, 2011). Commercially available impingers include AGI-30, the Burkard multistage sampler, the multi-orifice impinger (MOI), the multi-stage liquid impinger (MLI) and the Coriolis µ sampler.

The weaknesses associated with impingement include evaporation of liquid medium if sampled for long periods of time or during high temperatures, thus affecting the collection efficiency (Willeke *et al*., 1998). Other significant limitations involve the unsuitability for the collection fungal spores as most of the fungal spores are hydrophobic and can be lost in the liquid medium (Cartwright *et al.,* 2009). Impingers are time consuming and more expensive than Andersen impactors due to the necessity of sterilizing the impinger. By comparison the Andersen sampler can be easily sterilized with 70% ethanol and reused for sampling several times over a day.

1.5 Microbial Diversity of compost samples

Apart from bioaerosol related investigations, a number of studies have been conducted to access the microbial composition and abundance in the compost samples using molecular techniques. Some studies were carried out in open composting sites whereas others used in-vessel composting systems. In two separate studies, de Gannes *et al.* (2013 a & b) investigated the fungal and prokaryotic diversity of compost samples in three lingocellulosic compost systems using 454 pyrosequencing technology. In prokaryotic diversity analysis, eight phylum were the most abundant in sequences obtained including

Proteobacteria, Bacteroidetes, Firmicutes (Bacilli), Actinobacteria, Planctomycetes, Chloroflexi, Bacteria and *Archaea.* Concerning fungi, *Ascomycota* and Class *Sordariomycetes* were the most abundant (93%) and highest number of sequences accounting for species *Chaetomium funicola* (26%) and *Fusarium oxysporum* (12%). Fifteen pathogenic fungi were detected and seven pathogenic fungi were previously reported in the literature whereas eight were not identified in compost before. Hultman *et al.* (2010) and Partanen *et al.* (2010) in two separate studies, evaluated bacterial and fungal diversity at different stages of composting in large and small scale composting plants. Among fungi,

genera *Candida, Pichia* and *Dipodascaceae* dominated the mesophilic phase whereas, *Thermomyces, Candida* and *Rhizomucor* were dominant genera of the thermophilic phase. *Firmicutes, Actinobacteria, Bacteroidetes, Proteobacteria* and *Deinococcus-Thermus* were identified as dominant bacterial phyla in compost samples. To examine the relationship between physiochemical characteristics and bacterial community structure of compost samples, Karadag *et al.*, (2013) have shown dynamic bacterial diversity in different stages of the composting process is temperature dependent. In a laboratory scale study Ishii *et al.*, (2000) revealed microbial succession in composting process of garbage through DGGE analysis. The results showed that fermenting bacteria dominated the mesophilic phase, thermophilic Bacillus abundance in the thermophilic phase and furthermore complex microbial community distribution in the maturation phase.

1.6 Limitations in published literature on composting aerosols

Molecular and sequencing techniques used in these studies enhanced our knowledge about the microbial constituents of composting bioaerosols, although more in-depth research and understanding is needed to further elaborate the microbial ecology of bioaerosols from composting sites. This can be helpful to determine the acceptable levels of bioaerosols and formulating a buffer zone between composting sites and residential area.

To date most of the studies carried out on determining bioaerosol concentrations at composting sites lack data about background concentrations and community exposure to bioaerosols. There is limited information about the downwind dispersal of bioaerosols from composting facilities. Downwind concentration of bioaerosols and its comparison with the upwind and background concentration of bioaerosols can be utilized to quantify the risk of human exposure from composting facilities.

Furthermore, the overall microbial flora of bioaerosols in different phases of composting and do not provide deep information about the microbial processes (methanogenesis, acidogenesis and acetogenesis) that co-exist during composting. Detection and quantitation

of microbes via Q-PCR of functional genes encoding enzymes responsible for these microbial process is worthy of further investigation as it offers direct functional information. Although temperature and moisture content are important variables during the composting process; there is limited information about the influence of them on the emission and microbial signature of bioaerosols from composting facilities.

1.7 Aims of the study

The aims of this project were as follows:

- To investigate the composition and abundance of microbial communities present in bioaerosols from composting facilities.
- To examine the influence of temperature and moisture levels on the microbial diversity of composting bioaerosols.

The main objectives were as follows:

- To undertake a seasonal sampling regime of composting facilities utilising a personal sampler.
- To compare the levels of exposure to bioaerosols on workers and residents nearby composting sites.
- To investigate the effect of temperatures and moisture content on the microbial diversity of compost aerosols in laboratory settings.
- Detection and quantitation of specific bacterial and fungal pathogens through Q-PCR by identifying unique characteristic genetic signature of individual species.

The hypotheses tested in the research were as follow:

- The concentration of microorganisms in bioaerosols from compost sites is directly proportional to the proximity of the source (shredding, windrow turning) and long-distance dispersal of fungal communities will occur relative to bacterial communities.
- Seasonal variation in microbial community composition and abundance will occur whereby microbial abundance will increase in the summer months when temperatures increase compared to the winter.
- A difference in microbial community composition and abundance will occur during different time periods of corresponding moisture levels.

Chapter 2 Materials and Methods

2.1 Composting sites description

2.1.1 Birch Airfield composting facility, UK

Birch Airfield composting facility (Figures 2.1 A & B) is located near Colchester, Essex (51° 50′ 33″ N, 0° 46′ 50″ E) and has been functional since 2002. The site covers an approximate operational area of 26,241 m^2 and is surrounded by agricultural fields.

However, a sensitive receptor (solar power plant) also exists within 250 m of the site. Operating under an Environmental Agency permit, the plant accepts grass, trees, fruits and vegetable mainly from local authorities and recycling centres. However, the site does not offer composting services for kitchen waste as well as waste which is contaminated with non-biodegradable materials (plastic, metal, glass).

The composting facility uses open air windrow composting technology and is capable of recycling 40,000 tonnes organic waste annually (http://birchcomposting.com/about-us). On-site activities include shredding of green waste to produce windrows, turning of windrows and screening of compost material. At the start of the operation, waste is first shredded and approximately 4 * 4 meters (height * width) windrows of the shredded material are produced with the help of a mechanical shovel. To maintain the optimum composting conditions, windrows are turned weekly with a mechanical shovel. In the screening process, matured compost windrows are passed through a mechanical screener for the removal of oversized materials to achieve a size-based classification of compost product for gardeners (0-10 mm) and farmers (0-25 mm).

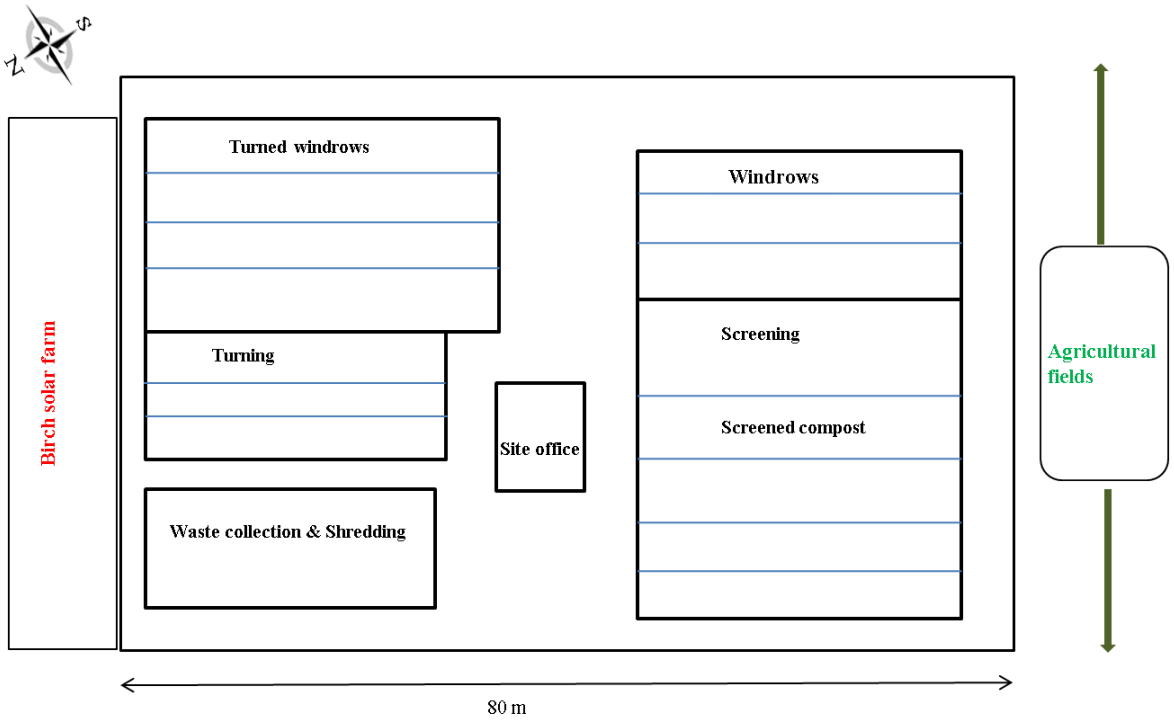

Figure 2.1 (A) Schematic diagram of Birch Airfield composting facility

Figure 2.1 (B) Location of Birch Airfield composting facility with reference to Colchester, UK

2.1.2 Waste Busters, Pakistan

Waste Busters (31.4035551° N; 74.3580674° E) is a municipal solid waste management organization, launched in 1996 and located in Lahore, Pakistan, (Figures 2.2 A & B). The facility obtains biodegradable waste through door to door collections of household garbage and green waste which mainly includes clippings from trees, grass, fruits and vegetables. In addition, the plant also uses animal waste i.e. cow dung, poultry waste and rice husk, for composting purposes. The site is capable of recycling approximately 20,000 tonnes organic waste per annum. However, it is situated in between a residential area and agricultural fields.

The waste management mainly involves collection of a wide range of wastes, separation of organic from inorganic matter and employs a windrow based method for composting. The collected waste undergoes mechanical and manual isolation in a segregation plant where inorganic waste is separated and sold to recycling industries; whereas organic matter is shredded and transferred to the composting area to produce about 2 meters (height x width) windrows with the help of mechanical shovels. Windrows are turned on a weekly basis and later screening of compost is done to obtain a desirable product. Before distribution of the compost, its quality is assessed for nutrient content, organic matter % and pH in a quality control laboratory established in the facility.

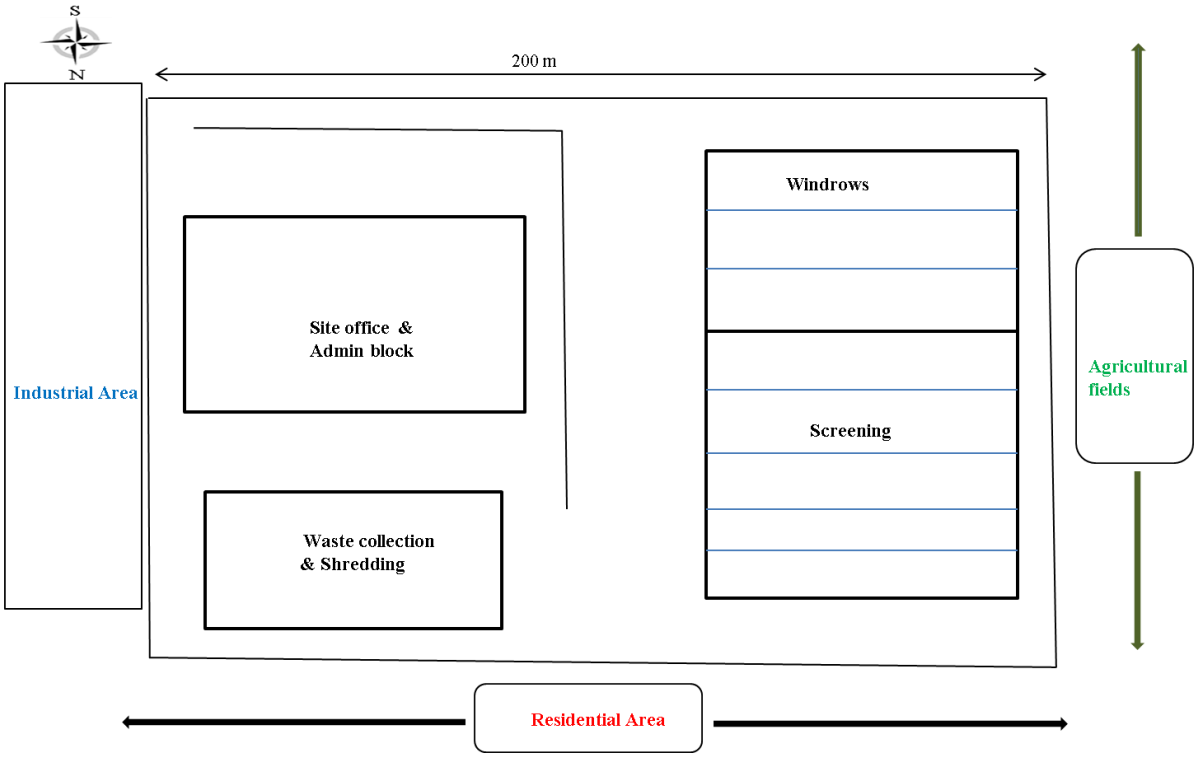

Figure 2.2 (A) Schematic diagram of Waste Busters composting facility

Figure 2.2 (B) Locations of the composting sites with reference to Lahore, Pakistan

2.1.3 Lahore compost (pvt) limited

Lahore compost facility (31.6107108° N; 74.3830088° E) is also situated in Lahore, Pakistan and has been operational since 2006 (Figures 2.3 & 2.2 B). Composting of municipal solid waste is carried out at a large scale composting site and receives organic components of the Municipal Solid Waste (MSW) of Lahore City, consisting of household kitchen waste and practices an open windrow composting technique. MSW is first placed on a moving conveyer belt for manual sorting of non-compostable material and then passed through a shredder to cut the remaining waste into fine pieces. Shredded material is then spread over a concrete pad with the help of mechanical caterpillar to create windrows (1.5 m in height and width) for the composting process and turned twice in a week for aeration. It takes 6 to 7 weeks to achieve mature compost windrows which later undergo mechanical screening to obtain the desirable compost product. The City District Government dumping site, industries and residential area are also located within 250 m of the site.

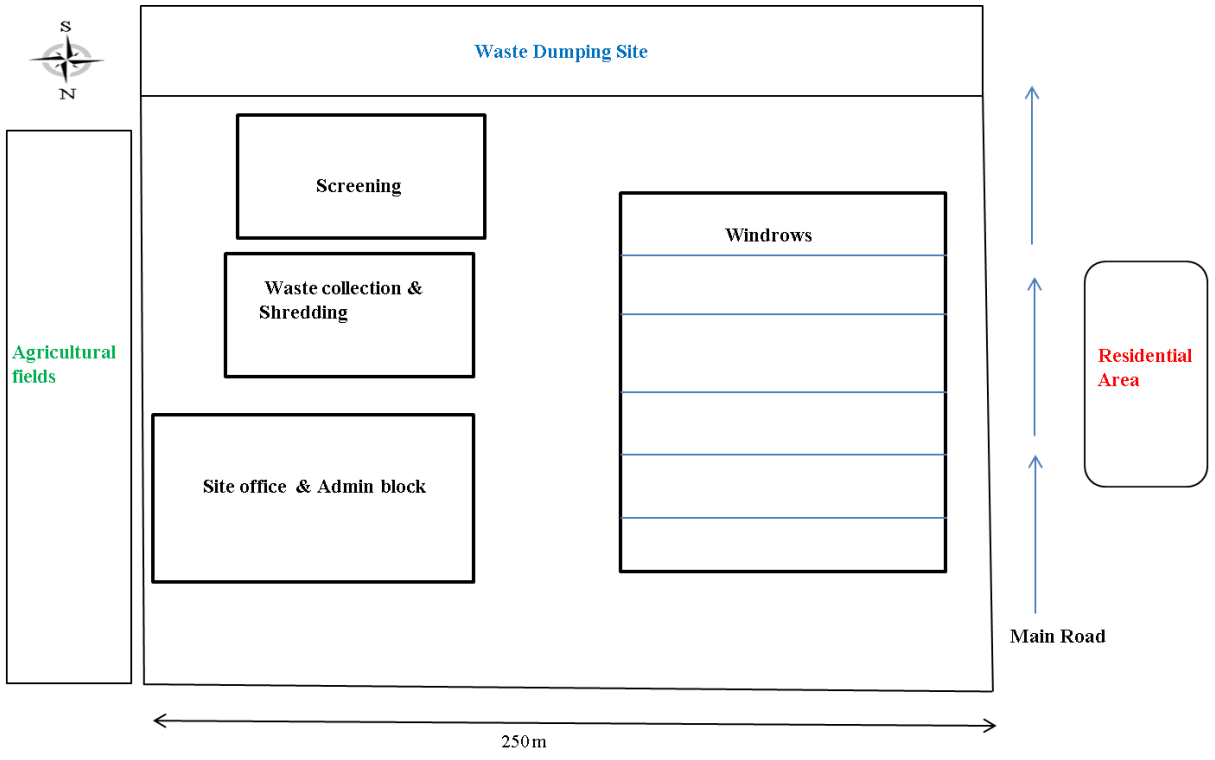

Figure 2.3 Schematic diagram of Lahore compost (pvt) limited facilit

2.1.4 Village Sampling

In addition to sampling at composting facilities, a minor sampling campaign was also carried out nearby manure heap in a village (32.860986° N; 72.759957° E) located in District Chakwal, Pakistan where traditional anaerobic composting is adopted to attain an organic supplement for better production of crops. Animal manure mixed with household grass and leaves are dumped at the backyard of houses on a daily basis that leads to the formation of a large pile which remains there throughout the year resulting in the anaerobic degradation of organic matter.

2.2 Sampling strategy

Sampling was carried out at increasing distances along the prevailing wind (parallel to the wind direction) and the distances of sampling points from the composting site were recorded through online GPS. A seasonal sampling campaign was undertaken with samples obtained in summer, autumn and winter and bioaerosols samples were taken in triplicates at each location i.e. onsite, upwind and downwind on each sampling regime (Table 2.1). The sampling arrangement and locations on different days were determined on the basis of wind direction, site operations and access limitations (fig 2.4 & 2.5). Meteorological data were recorded on each sampling day with a Rotronic CP11 instrument (Rotronic AG, Switzerland) and Kestrel® 4000 weather station (Richard Paul Russell Ltd, UK).

Table 2.1. Bioaerosols sampling schedule from composting sites

Composting sites	Seasons	Sampling dates
Birch Airfield	Summer	21st July, 2015
Birch Airfield	Autumn	30th September, 2015
Birch Airfield	Winter	9th December, 2015
Waste Buster	Autumn	7th October, 2015
Lahore compost (pvt)	Autumn	9th October, 2015

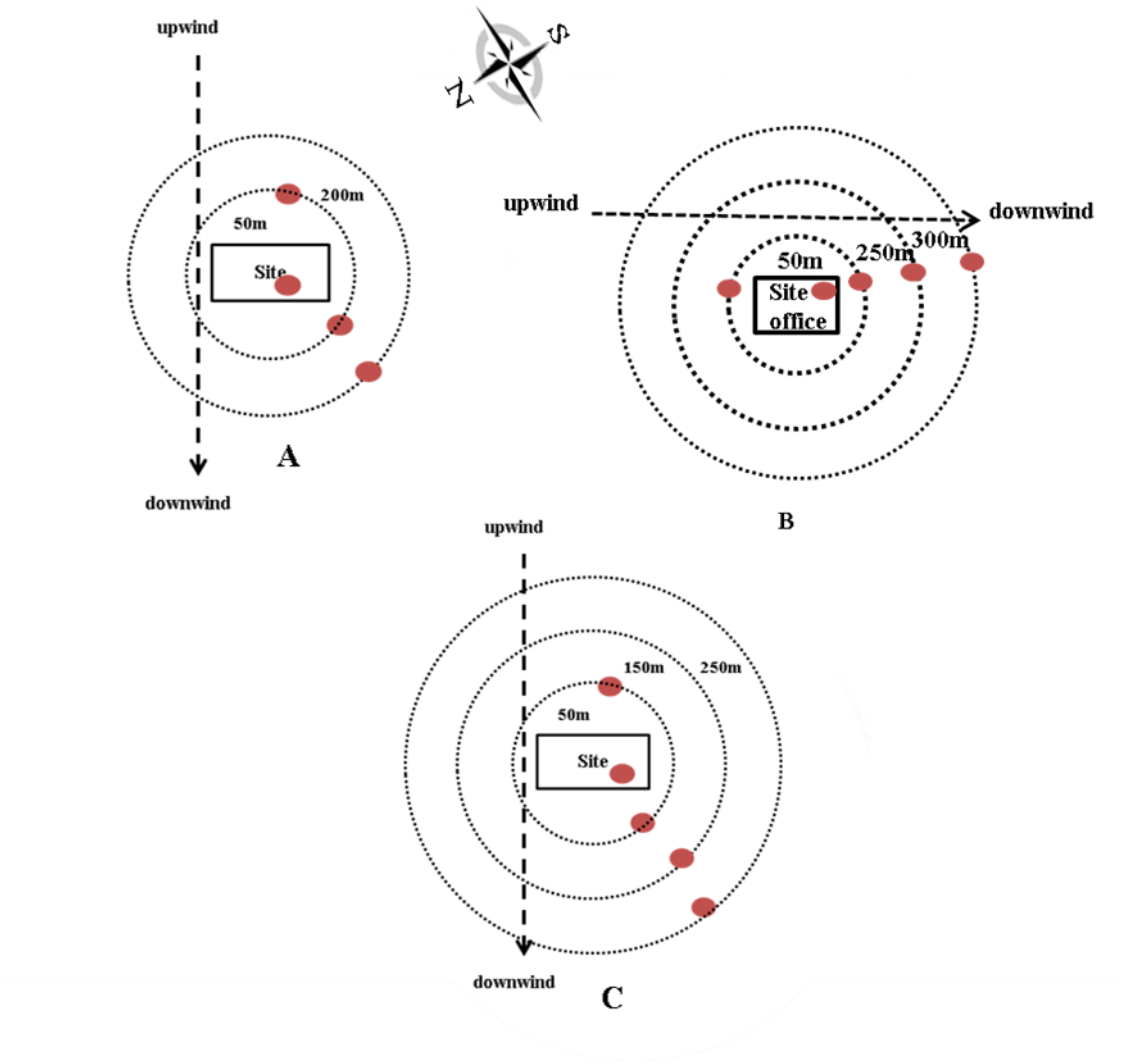

Figure 2.4 Bioaerosol sampling arrangement in summer (A), autumn (B) and winter (C) at the Birch Airfield composting facility

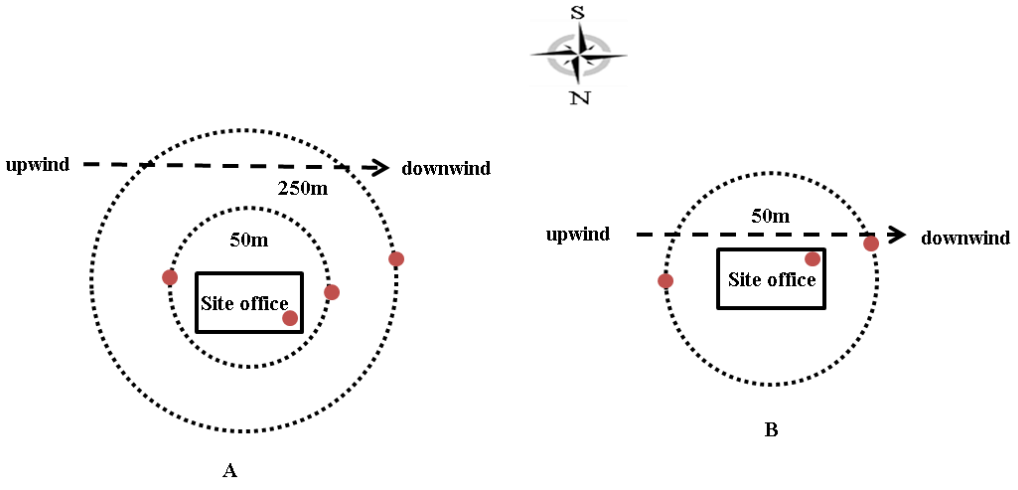

Figure 2.5 Bioaerosols sampling arrangement at the Waste Buster composting facility (A) and Lahore compost facility (B), Pakistan

2.3 Sampling approach

2.3.1 Compost sample collection

Approximately 150-200g of two-week old shredded compost (10 cm depth) was aseptically collected and placed in a sterile translucent high density polyethylene jar (87 mm x 117 mm x 86 mm) using a sterile spatula and stored at -20°C. Samples were collected from the Birch Airfield composting facility (fig 2.1).

2.3.2 Bioaerosol collection

Bioaerosols were obtained using an IOM personal inhalable sampler attached with Universal air pump (SKC Inc.). This sampler impacts air onto the surface of a filter inserted in a 25 mm filter cassette at fixed rate (2 l / min) for 30 minutes (fig 2.6). The filters used were sterile 25 mm, 0.4 µm polycarbonate filters (Sartorius). Filter cassettes were stored on ice and transported to the laboratory. Approximately 5g – 10g of compost samples were also collected in sterile universal tubes on each sampling day.

A B

Figure 2.6 Sampling of bioaerosols onsite (A) and downwind (B) of the composting facility.

2.4 Experimental design of compost boxes used in the laboratory scale study

Three perspex boxes (l x h x w: 400 mm x 250 mm x 250 mm) were utilized in this research (Figure 2.7 & 2.8). Cylinders of 125 mm diameter, attached with a rotating handle, were placed in the boxes in which shredded compost of almost 1 week old was employed. To investigate the effect of temperature, compost having identical moisture content i.e. 60% was placed in the boxes at three different temperatures i.e. 12°C, 23°C and 33°C.

Moisture content of the compost was set using a pressure plate extractor (ICT, 1500F 15 Bar) before placing the compost within the cylinders. In order to assess the influence of moisture content (humidity) on bioaerosols' generation the moisture content of all the three boxes were adjusted at 40%, 60%, 80% and placed in a constant temperature room at 33°C. A 5 mm diameter hole was also made at the top of the boxes to obtain measurements of humidity.

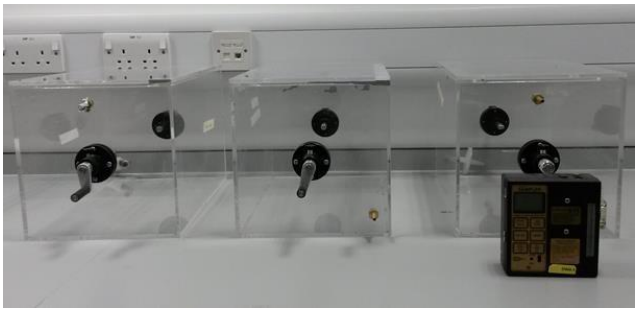

Figure 2.7 Perspex boxes utilized in the laboratory scale composting experiment.

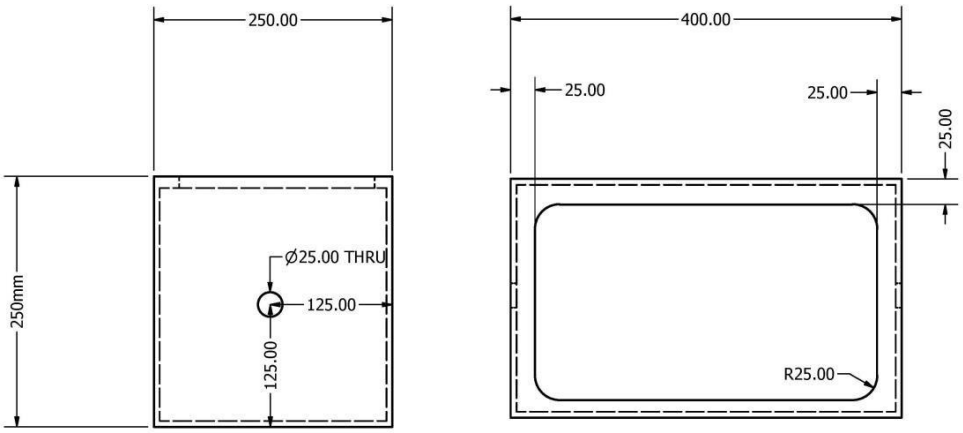

Figure 2.8 Layout of compost box in figure 2.7 (dimensions in mm).

2.4.1 Sampling strategy

Sampling was undertaken from the boxes on weekly basis and three samples were taken consecutively in each sampling operation on respective days. Before each sampling, the compost cylinder was rotated thrice (manually) for the generation of aerosols. Bioaerosol collection was carried out using an in-line Delrin Filter Holder (Sartorius), attached with SKC Universal air pump. This sampler impacts air onto the surface of filter inserted in a

stainless steel screen (25 mm) at fixed rate (2 l/min) for 30 minutes. The filters employed in this study were sterile 25 mm, 0.4 µm polycarbonate filters (Sartorius). After each sampling interval, the filters were stored at -20°C for subsequent laboratory analysis. Humidity measurements were recorded on each sampling day with a CP11 handheld instrument (Rotronic).

2.5 DNA Extraction

DNA was extracted by transferring the whole filters into sterile bead beating tubes containing 0.5 g of 0.1 mm zirconia silica beads. Cells on filters were lysed using 0.5 ml extraction buffer [1MTris-HCl pH=8, 0.5M EDTA, 10% (w/v) SDS, 5M NaCl] followed by bead beating for 10 min. Tubes were incubated for 30 min at 70°C (Ghatak et al., 2013). 0.5ml of Phenol/Chloroform/Isoamyl alcohol (25:24:1 (v/v) pH 8.0) was added in the tubes and centrifuged (MSE MicroCentaur) at 18928 g for 5 min. The upper aqueous layer was removed into new sterile Eppendorf tubes and precipitated with 500 µl of isopropanol and 2.5 µl glycogen for 60 min. Tubes were then subjected to centrifugation at 18928 g for 25 min and DNA pellets were obtained and washed with 70% (v/v) ethanol. Total DNA was air dried at room temperature for 10 min and resuspended in 35µl sterile distilled water and stored at -20°C.

2.6 PCR amplification of bacterial/archaeal 16S rRNA gene and the fungal ITS region

The PCR primers used in this study are presented in Table 2.1. The bacterial 16S rRNA gene PCR primers and cycling conditions used in this study were as previously described (Muyzer *et al.,* 1993). PCR amplification of archaeal 16S rRNA genes used the PCR primers and cycling conditions as described by Kuroda *et al.,* (2015). PCR amplification of the fungal Intergenic Spacer (ITS2) Region was performed as follow: an initial denaturation step of 94 °C for 3 min, followed by 32 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 45 s and extension at 72 °C for 50 s and final extension at 72

°C for 7 min. Each 50 µl reaction contained 2 µl of template DNA (15-20ng/µl), 0.4 µM of

both forward and reverse primers (1 µl), 25 µl of MyTaq Red Mix, 2x (Bioline).

Amplification was performed in a Gene Amp® PCR system 9700 Thermocycler (Applied Biosystems) and targeted sequence was confirmed through agarose gel electrophoresis (1% w/v in 1 x TAE at 110 V), stained with ethidium bromide (4µg/ml) and visualized under an UV illuminator (Innotech AlphaImager EP).

Table 2.2 Primers utilized in this research.

Primer name	Sequence ($5^{/}$ - $3^{/}$)	Target	Application	References
341F-GC clamp	CCTACGGGAGGCAGCAG	16S rRNA (Bacteria)	DGGE	Muyzer *et al.,* (1993)
534R	ATTACCGCGGCTGCTGG	16S rRNA (Bacteria)	DGGE	Muyzer *et al.,* (1993)
ITS3F-GC	GCATCGATGAAGAACGCAGC	ITS region (Fungi)	DGGE	White *et al.,* (1990)
ITS4R	TCCTCCGCTTATTGATATGC	ITS region (Fungi)	DGGE	White *et al.,* (1990)
516F	TGTCAGCCGCCGCGGTAATACCAGC	16S rRNA (Archaea)	DGGE	Kuroda *et al.*,(2015)
806R	GGACTACCAGGGTATCTAAT	16S rRNA (Archaea)	DGGE	Kuroda *et al.*,(2015)
341F	CCTACGGGAGGCAGCAG	16S rRNA (Bacteria)	Q-PCR	Muyzer *et al.,* (1993)
534R	ATTACCGCGGCTGCTGG	16S rRNA (Bacteria)	Q-PCR	Muyzer *et al.,* (1993)
86F	GCCCGCCGTTTCGAC	ITS region (*A. fumigatus*)	Q-PCR	Walsh *et al.,* (2011)
221R	CCGTTGTTGAAAGTTTTAACTGATTAC	ITS region (*A. fumigatus*)	Q-PCR	Walsh *et al.,* (2011)
86F	TGTGGTGGGGTGGATGAGT	16S rRNA (*S. rectivirgula*)	Q-PCR	Schäfer *et al.,* (2011)
183R	ACCATGCGGCAGAATGTCCT	16S rRNA (*S. rectivirgula*)	Q-PCR	Schäfer *et al.,* (2011)
ITS3F	GCATCGATGAAGAACGCAGC	ITS region (Fungi)	Sequencing	White *et al.,* (1990)
ITS4R	TCCTCCGCTTATTGATATGC	ITS region (Fungi)	Sequencing	White *et al.,* (1990)
341F	CCTACGGGAGGCAGCAG	16S rRNA (Bacteria)	Sequencing	Muyzer *et al.,* (1993)
805R	GACTACHVGGGTATCTAATCC	16S rRNA (Bacteria)	Sequencing	Klindworth *et al.,* (2012)

2.7 Quantitative PCR of bacterial 16S rRNA gene and fungal ITS region

PCR products of target genes were generated utilizing suitable primers and purified using QIAquick PCR purification kit (Qiagen) Standard curves were created using purified PCR

products of the target genes i.e. 16S rRNA and ITS region. The purified amplicons were quantified using a Nanodrop® ND- 3300 spectrophotometer. The purity of the standards were determined by calculating the absorption ratio A_{260}/A_{280} (ratio of 1.8 represents pure DNA) prior to the generation of standard curve using a dilution series for each standard containing (10^1 - 10^7) target genes/µl. The copy number of the target sequence was calculated assuming 660 Da molecular mass for double stranded DNA using following formula:

Copy number = 6.022 x 10^{23} (copies/mole) x standard concentration (g/µl) / MW (g/mol).

Standards were amplified along with the unknown samples and C_t values for each template were determined. A linear regression line, produced from the C_t values of the standards, was plotted against the log of their initial concentration. The construction of standard curve and C_t values were carried out utilizing ABI Prism 7000 sequence detection software (Applied Biosystems) and the original numbers of unknown target templates were then quantified by comparison of their C_t values with that of standard curve (Osborn and Smith, 2005). The qPCR efficiency for the bacteria and *S. rectivirgula* quantification systems was 96% and 95% respectively with a regression coefficient value (r^2) above 0.98. For the *A. fumigatus* quantification system, *E* was between 87% and 97% and R2 always above 0.98.

Reactions were carried out on CFX96 real time PCR detection System (BIO-RAD) with cycling conditions of Initial denaturation at 95°C for 3 min; followed by 39 cycles of denaturation and annealing at 95°C and 60°C for 10sec and 30sec respectively. Each 20 µl of reaction contained 1 µl of template, 100Nm of each primers and 10 µl of 2x SensiFAST™ SYBR® Green master mix (Bioline). Negative controls samples containing no DNA template were also included in all the reactions to detect any contamination. The verification of the amplification of the double stranded DNA was attained by running a dissociation protocol at the end of each run.

2. 8 Denaturing Gradient Gel Electrophoresis (DGGE) of bacterial/archaeal 16S rRNA gene and fungal ITS region

PCR amplified products were analysed through DGGE utilizing the Bio-Rad D Code system. Gels containing 8% (w/v) polyacrylamide were subjected to electrophoresis (37:1 acrylamide/bis-acrylamide) in 1 x (TAE) buffer (40mM Tris-acetate, 1mM di-sodium-EDTA, pH 8.0) at constant 55 V and 60°C for 14 hours. The denaturing gradient used was from 40% to 60% (w/v) where 100% denaturant comprised of 7M urea and 40% (v/v) formamide. After the completion of electrophoretic run, gels were silver stained as described previously (Nicol *et al.*, 2005).

2.9 Statistical analysis

Data presented in chapters 3-5 were analysed by determining arithmetic mean values. ANOVA analysis was performed on environmental and Q-PCR data with post hoc Tukey's Honest Significance Difference (HSD) test to determine the statistically significant differences. The microbial population fingerprint produced through DGGE was converted into binary matrix and Microsoft Excel (2010) was utilized as a statistical tool to perform these tasks. Cluster analysis from the binary data was determined by calculating the distance matrix on the basis of Jaccard's distance and then generating clusters utilizing hierarchical clustering method. The statistical tool to perform this task was RStudio version 3.3.1 (2016).

Chapter 3 Seasonal study of the microbial diversity of bioaerosol emissions from a composting facility, UK

3.1 Introduction

Although on-site operational activities are regarded as the significant source of bioaerosol emissions, meteorological conditions such as, temperature and wind speed have been shown to affect the dispersal of bioaerosols from composting sites (Wéry, 2014).

Seasonal meteorological influences are suggested to modify the concentration and composition of compost aerosols. Studies have indicated higher concentrations during summer and autumn periods with a decline in winter (Nielsen *et al,.* 1995; Recer *et al.,* 2001; Schlosser *et al.,* 2009). However, the variations in the composition and concentration of bioaerosols have also been suggested to be influenced by onsite activities such as, turning, shredding and screening (Recer *et al.,* 2001).

The dispersal of microbial spores is affected by changes in the tension and bonding forces between the spores and the surface. The alteration of these forces, affected by temperature and moisture, of both compost surface and adjacent air, result in the emissions of microorganisms (Gorny, 2004; Jones & Harrison, 2004).

It has been indicated that bioaerosols can be dispersed up to 1000m from sites under certain wind conditions (Albrecht *et al.,* 2008). Temperature and relative humidity are suggested to affect the cultivability of *A. fumigatus*; (Ho *et al.,* 2005); whereas high temperatures, desiccation and UV rays result in reducing the viability of aerosolized bacteria (Harrison *et al.,* 2005; Lighthart & Mohr, 1987). The dispersal and survival of bioaerosols in the atmosphere are effectively dependent on these parameters. For example airborne microorganisms may become inactivated through desiccation, UV exposure and warm temperatures (Wery, 2014). However, there is scarcity of data about the characterization of these parameters. There is a complexity about the interaction of bioaerosols and environmental settings depending on the physiochemical properties of bioaerosols i.e. hydrophobicity, aerodynamic diameter, oxygen sensitivity, and electrical charge.

These properties result in variation of interactions with parameters, such as humidity, temperature, winds and UV radiation. Further to this, numerous studies (Lacey & Crook, 1988; Kampfer *et al.,* 2002; Swan *et al.,* 2003) have suggested health hazards due to the presence of pathogenic species in compost aerosols. Prolonged exposures of certain pathogens represent risks of serious infections in workers and among these *Aspergillus fumigatus* and *S. rectivirgula* are of major importance. *Aspergillus fumigatus* can cause invasive aspergillosis in immuno- deficient subjects (Taha *et al.*, 2007); whereas, *S. rectivirgula* can stimulate hypersensitivity induced pneumonitis and one of the major agents of extrinsic allergic alveolitis (Duchaine *et al.*, 1999; Wery, 2014). *Saccharopolyspora sp.* has also been previously used as a microbial indicator for monitoring the abundance of airborne microorganisms in bioaerosols from composting plants (Le Goff *et al.,* 2011; 2012).

The aim of the study was to quantify the abundance of specific pathogenic microorganisms in bioaerosols emitted from the composting facilities and determine the impact of seasonal conditions on bioaerosol dispersion. It is hypothesised that there will be a higher abundance of bacterial pathogens in bioaerosols close to site operations whilst fungal pathogens will be more abundant further away from the point source of contamination - compared to bacterial communities. Seasonal variation will also influence microbial abundance, whereby there will be greater microbial abundance in the summer months when temperatures are higher compared to winter. These findings will contribute to a better understanding of the seasonal climatic influences on the composition and abundance of bioaerosols emitted from composting sites

3.2 Results

A summary of sample types and ID, onsite activities, locations and meteorological data obtained during the seasonal sampling regime at the UK composting site is given in Table 3.1 and Figure 3.1.

Table 3.1 Description of bioaerosol sampling operation during the seasonal sampling campaign at Birch Airfield composting facility

Seasons	Sample type	Sample ID	Onsite activities during sampling	location	Wind speed m/s	Humidity %	Temperature °C
Summer	Air	Up- a, b, c	Screening Turning	50 m upwind	4.5	49	21.6
	Air	On- a, b, c	Screening Turning	onsite	5.3	43	22.3
	Air	D1- a, b, c	Turning	50 m downwind	5.5	41	23.1
	Air	D2- a, b, c	shredding	200 m downwind	5.1	42	22.8
	Compost	C-T1	-	-	-	-	-
Autumn	Air	Up- a, b, c	Screening shredding	50 m upwind	3.7	51	21.5
	Air	On-a, b, c	Screening shredding	onsite	4.8	73	16.4
	Air	D1- a, b, c	Screening shredding	50 m downwind	4.2	73	16.3
	Air	D2- a, b, c	Turning	250 m downwind	4.5	69	17.7
	Air	D3- a, b, c	Turning	300 m downwind	5.1	70	17.4
	Compost	C-T-2	-	-	-	-	-
Winter	Air	Up- a, b, c	Turning, shredding	50 m upwind	3.2	82	10.2
	Air	On- a, b, c	Screening	onsite	4.4	78	11.5
	Air	D1- a, b, c	Shredding	50 m downwind	3.5	84	9.1
	Air	D2- a, b, c	Turning	150 m downwind	4.6	83	8.4
	Air	D3- a, b, c	Screening Turning	250 m downwind	3.8	83	8.1
	Compost	C-T-3	-	-	-	-	-

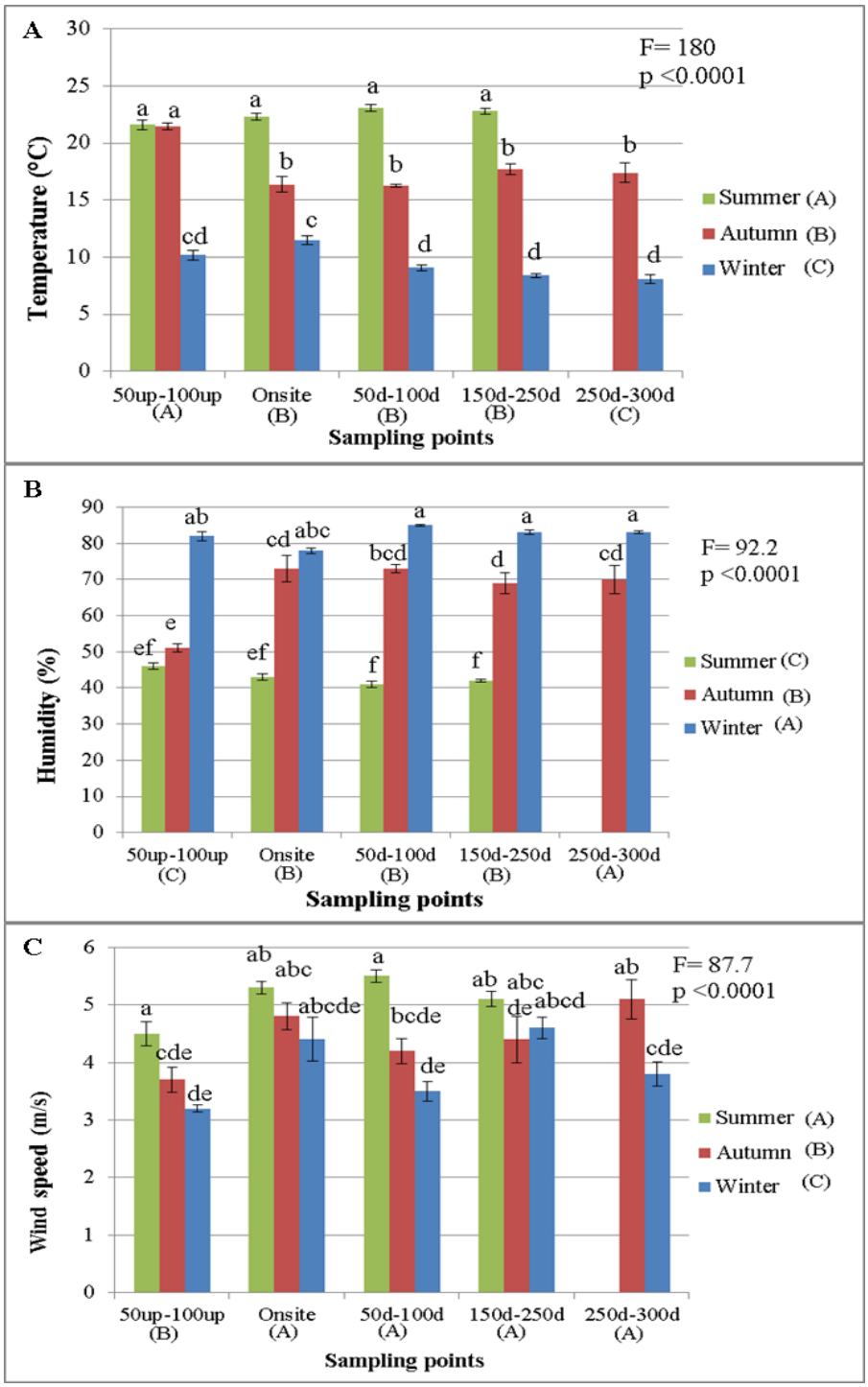

Figure 3.1 Temperature (A), humidity (B) and wind speed (C) recorded during the seasonal sampling campaign. 50up – 100up = 50m – 100 m upwind; 50d – 100d = 50m – 100 m downwind; 150d – 250d = 150d – 250 m downwind; 250d – 300d = 250m downwind – 300m downwind of the composting site. Error bars represent standard error of mean (*n*=6).Significant differences are shown by ANOVA and Tukey's HSD. Different letters in brackets after seasons, sampling points or above bars indicate significant differences at 0.05 confidence interval.

3.2.1 Quantification of total bacteria over a seasonal cycle

The concentration of total bacteria over a seasonal cycle was determined and shown to decrease with increasing distance from the composting facility with maximum and minimum abundance acquired at onsite and 50 m upwind respectively (Fig 3.2). The mean abundances of bacteria during different seasons are summarized in Table 3.2.

In summer, an increase of 3 orders of magnitude was observed between the 50 m upwind and onsite locations; whereas an increase of 1 order of magnitude was observed between onsite and 200 m downwind of the site. In autumn, an increase of 1 order of magnitude was observed between the 50 m upwind, 250 m and 300 m downwind and onsite locations (Fig 3.2). In winter, an increase of 1 order of magnitude was observed between the onsite and 50 m upwind and 250 m downwind locations of the site (Fig 3.2).

Table 3.2 Quantification of bacteria in bioaerosols emitted from Birch Airfield composting facility in summer, autumn and winter.

Sampling points	Summer	Autumn	Winter
50up-100up	7.6×10^7	6.01×10^7	8.2×10^7
Onsite	5.5×10^{10}	1.2×10^9	8.2×10^8
50d-100d	1.3×10^{10}	6.3×10^8	3.7×10^8
150d-250d	9.9×10^9	6.8×10^7	4.5×10^8
250d-300d		6.7×10^7	8.8×10^7
Compost	3.2×10^9	1.1×10^9	8.5×10^8

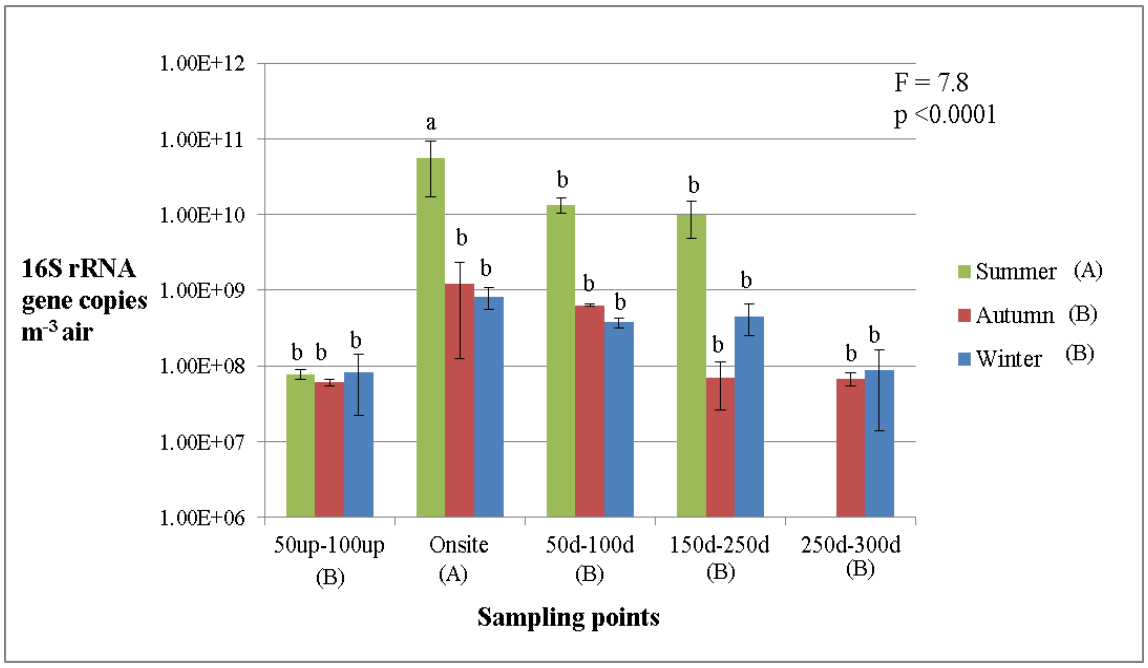

Figure 3.2 Abundance of bacteria in bioaerosols emitted from Birch Airfield composting facility in summer, autumn and winter. 50up – 100up = 50m – 100 m upwind; 50d – 100d = 50m – 100 m downwind; 150d – 250d = 150d – 250 m downwind; 250d – 300d = 250m downwind – 300m downwind of the composting site. Columns with similar superscripts are significantly different from each other (p-value < 0.05). Error bars represent standard error of mean (*n*=3). Significant differences are shown by ANOVA and Tukey's HSD. Different letters in brackets after seasons, sampling points or above bars indicate significant differences at 0.05 confidence interval.

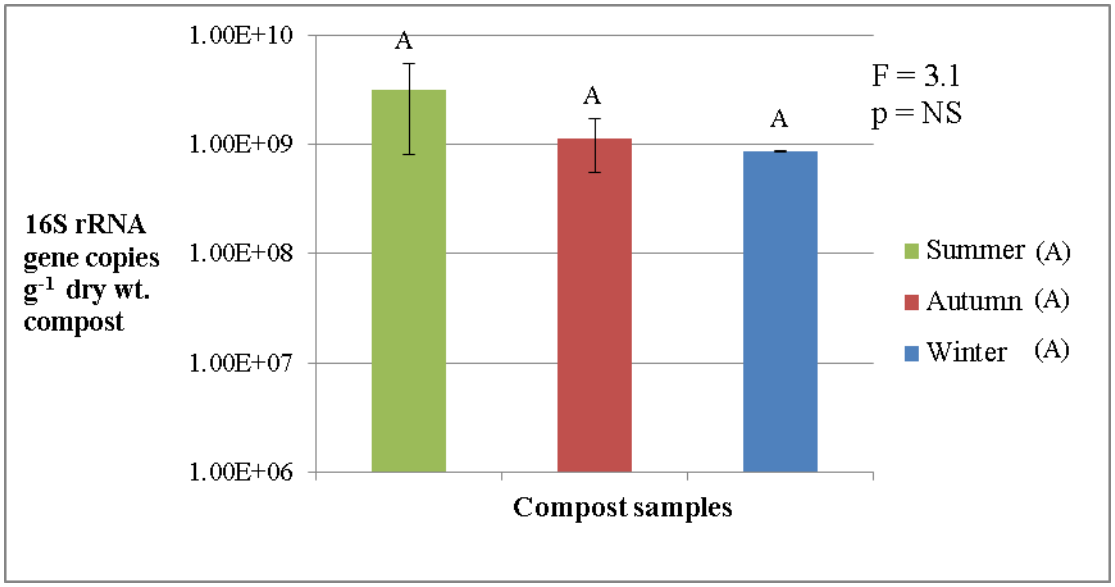

Figure 3.3 Abundance of bacteria in compost in different seasons from Birch Airfield composting facility. Error bars represent standard error of mean (*n*=3). Similar letters in brackets after seasons or above bars indicate non- significant differences at 0.05 confidence interval.

3.2.2 Quantification of *Saccharoployspora rectivirgula* over a seasonal cycle

In the summer, the mean abundance of *Saccharopolyspora rectivirgula* onsite was maximum that reduced at 50 m downwind; however, a slight decrease in the abundance was observed at 200 m downwind. Conversely, a drop in the abundance was observed at 50 m upwind. An increase of 2 order of magnitude was observed between the upwind and onsite and downwind locations of the facility (Table 3.3) (Fig 3.4).

In autumn, 2 orders of magnitude increase was observed between onsite and 50 m upwind, 250 m and 300 m downwind samples; whereas 1 order of magnitude was detected between onsite and 50 m downwind of the composting site (Table 3.3) (Fig 3.4). In winter season, the increase in the abundance at onsite was increased 1 order of magnitude between the

onsite as compared to upwind and downwind samples (Table 3.3) (Fig 3.4). In the summer, the correlation matrix of mean abundance of *S. rectivirgula* with meteorological variables indicated temperature and wind speed exhibited a positive linear relationship with the abundance of *S. rectivirgula*; whereas, humidity indicated negative linear relationship with *S. rectivirgula.* However, all the correlations of abundance of bioaerosols and climatic variables were found insignificant (Appendix table 3.2). In autumn, the correlation matrix of abundance of *S. rectivirgula* with meteorological variables indicated that humidity and wind speed were positively correlated (linear relationship) with the abundance of *Saccharopolyspora rectivirgula*; whereas, temperature represented a negative linear relationship (Appendix table 3.3).

Table 3.3 Quantification of *Saccharoployspora rectivirgula* in bioaerosols emitted from Birch Airfield composting facility in summer, autumn and winter.

Sampling points	Summer	Autumn	Winter
50up-100up	8.4×10^6	3.1×10^6	2.7×10^7
Onsite	8.7×10^8	2.3×10^8	3.2×10^8
50d-100d	4×10^8	2×10^7	1.4×10^7
150d-250d	2.9×10^8	5.2×10^6	1.5×10^7
250d-300d		2.6×10^6	1.9×10^7
Compost	2.1×10^7	2.72×10^7	1.5×10^7

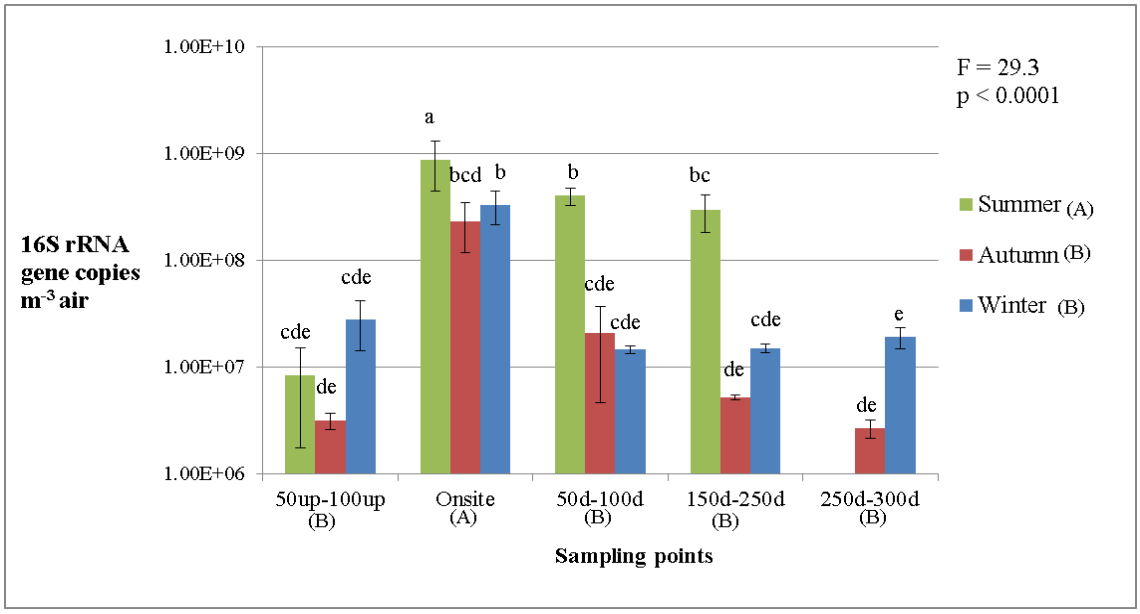

Figure 3.4 Abundance of *Saccharoployspora rectivirgula* in bioaerosols emitted from Birch Airfield composting facility in summer, autumn and winter. 50up – 100up = 50m – 100 m upwind; 50d – 100d = 50m – 100 m downwind; 150d – 250d = 150d – 250 m downwind; 250d – 300d = 250m downwind – 300m downwind of the composting site. Error bars represent standard error of mean (*n*=3). Significant differences are shown by ANOVA and Tukey's HSD. Different letters in brackets after seasons, sampling points or above bars indicate significant differences at 0.05 confidence interval.

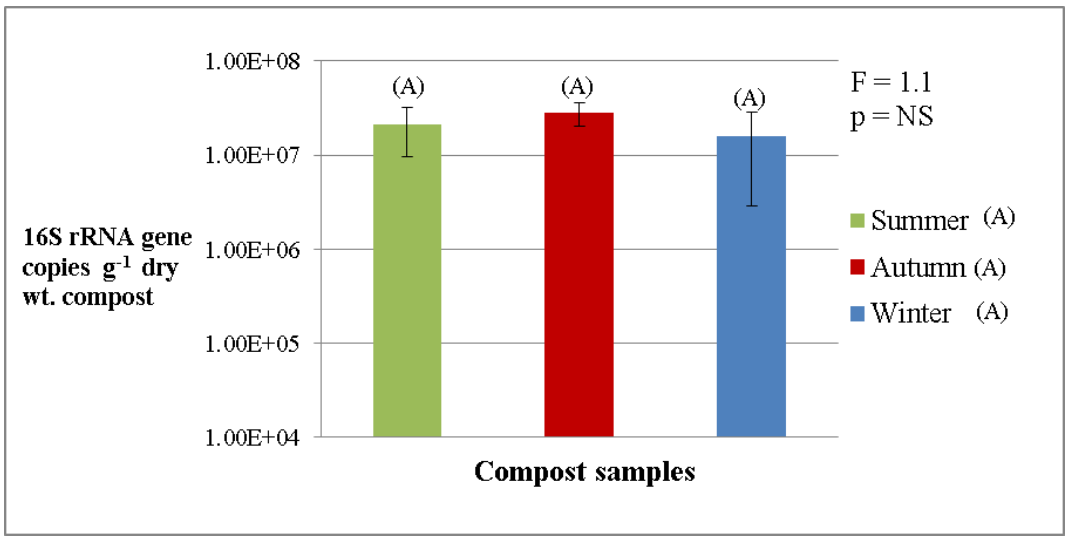

Figure 3.5 Abundance of *Saccharoployspora rectivirgula* in compost in different seasons from Birch Airfield composting facility. Error bars represent standard error of mean (*n*=3). Similar letters in brackets after seasons or above bars indicate non- significant differences at 0.05 confidence interval.

3.2.3 Bacterial community analysis over a seasonal cycle

PCR-DGGE analysis of bacterial 16S rRNA gene showed a varying microbial community dynamics between the compost samples and the different locations across the site during the summer (Fig 3.6a). The bands represented in “A2-a – A2-c” were found in the compost but absent in the aerosol samples. Some bands were found in all samples (A1a- A1-e). Hierarchal cluster analysis of the band patterns indicated 50 % similarity of bacterial signatures between onsite and 50 m downwind positions (The bands shown in “A5-b” were fingerprints that were similar onsite, 50 m and 200 m downwind); whilst 40% similarity was characterized between onsite and 200 m downwind locations (Fig 3.6b). DGGE profiles of the compost sample indicated almost 20% similarity among onsite and downwind samples. However, a significant dissimilarity of bacterial community structure was observed at upwind locations. The bands in “A3-a” showed fingerprints in the upwind samples, while “A6-a” showed profiles found in the 50 m and 200 m downwind (Fig 3.6b).

In the autumn season, DGGE analysis of 16S rDNA PCR amplified products indicated a similar pattern of bacterial species distribution between compost and onsite as compared with downwind and upwind locations (Fig 3.7a). Cluster analysis revealed 40% similarity between compost and onsite aerosol microbial structure. However, some bands only appeared in the onsite samples as shown in “C2-a- C2-c”. Similar fingerprint distribution was observed in the onsite and downwind samples as well as compost and downwind samples as indicated in the “C3a-C3-b” and “C4a-C4-b”. Bacterial community obtained at 50 m downwind was divergent as compared with the onsite and compost communities (several bands appeared in the 50 m downwind samples were not found in other samples as indicated by “C6-a-C6-d”); although in 150 m and 250 m downwind the bacterial signature was 70% similar with each other (Fig 3.7b). However, the overall microbial community dynamics observed at downwind locations indicated almost 30% similarity with the compost and onsite communities. Conversely, 50 m upwind microbial distribution showed a higher dissimilarity comparing onsite, downwind and compost communities. Some of the

fingerprints showed high abundance at 50 m upwind samples i.e. “C7-a-C7- b” (Fig 3.7b).

PCR-DGGE analysis of 16S rRNA gene of compost and aerosols obtained during the winter showed greater microbial community dynamics onsite compared with downwind and upwind locations (Fig 3.8a). “E1-a- E1-c” represented band profiles appeared in the compost and aerosols samples whereas, “E2a-E2b” indicated the incidence of similar fingerprints in the bioaerosol samples while some only appeared in the onsite samples i.e. “E3a- E3d”. However, the banding arrangement showed a decrease in bacterial diversity observed at 50 m downwind with a further increase at 150 m downwind. Hierarchal cluster analysis revealed that the microbial structure of the compost sample was highly dissimilar with the aerosol samples collected onsite, upwind, and downwind (Fig 3.8b). The microbial signature of the bioaerosols obtained onsite and upwind indicated almost 50-60% similarity with each other. The overall bacterial population structure observed at downwind positions displayed 30% similarity with onsite and upwind microbial diversity. “E4a- E4b” represented that some fingerprints appeared in 250 m downwind and 50 m upwind were high in abundance as compared with the band profiles of 50 m and 150 m downwind (Fig 3.8b).

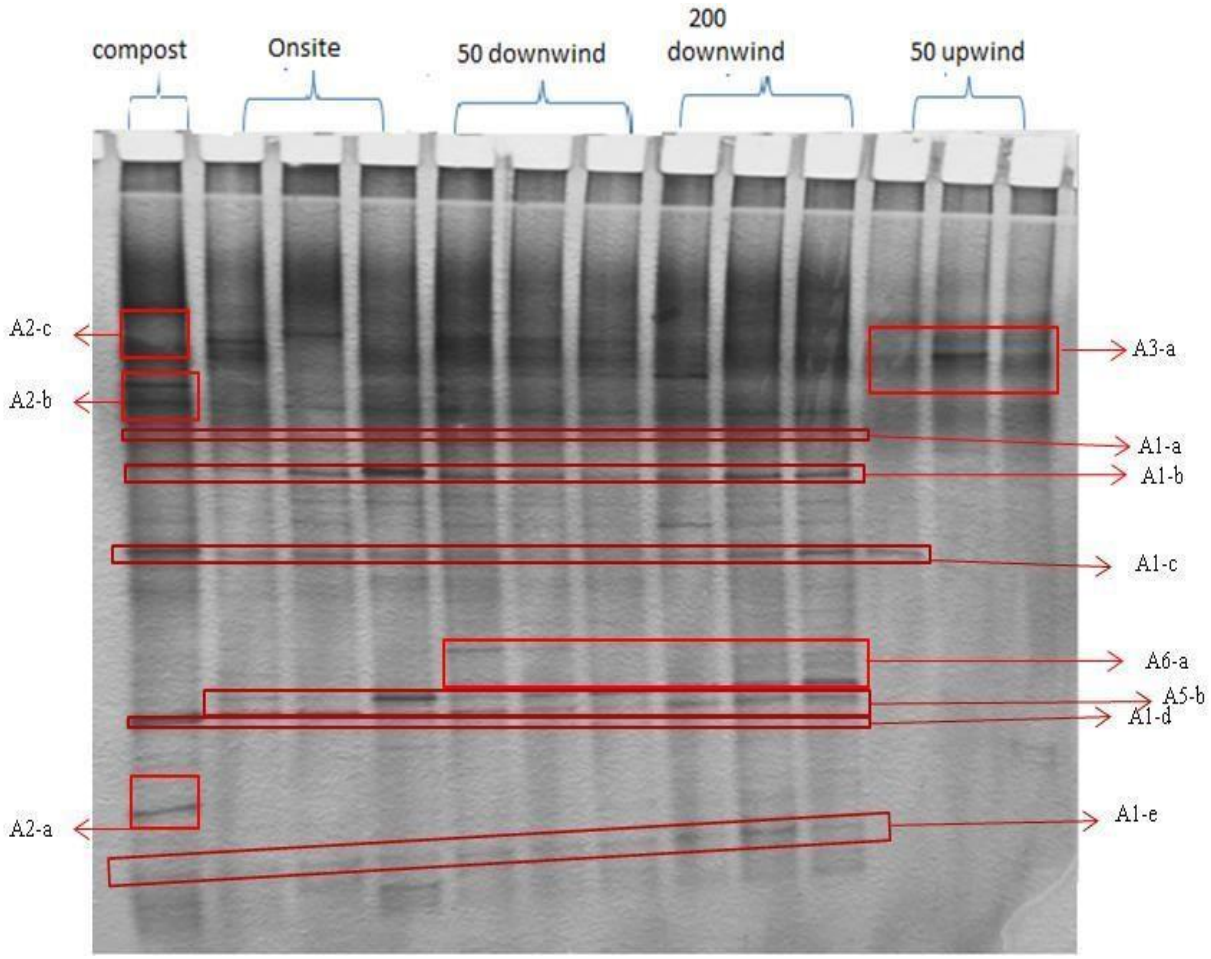

Figure 3.6 (a) DGGE profiles of bacterial 16S rDNA PCR amplicons of compost and bioaerosols from the summer season.

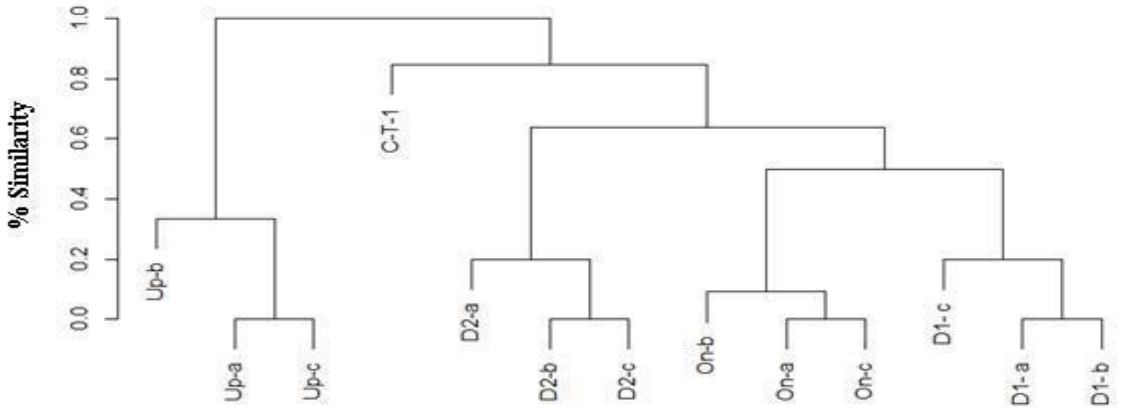

Figure 3.6 (b) Cluster dendrogram of the banding pattern in figure 3.6 (a) using a complete linkage (jaccard cofficient). C-T-1 = compost; Onsite = On - a, b, c; 50 m downwind = D1- a, b, c; 200 m downwind = D2- a, b, c; 50 m upwind = UP- a, b, c.

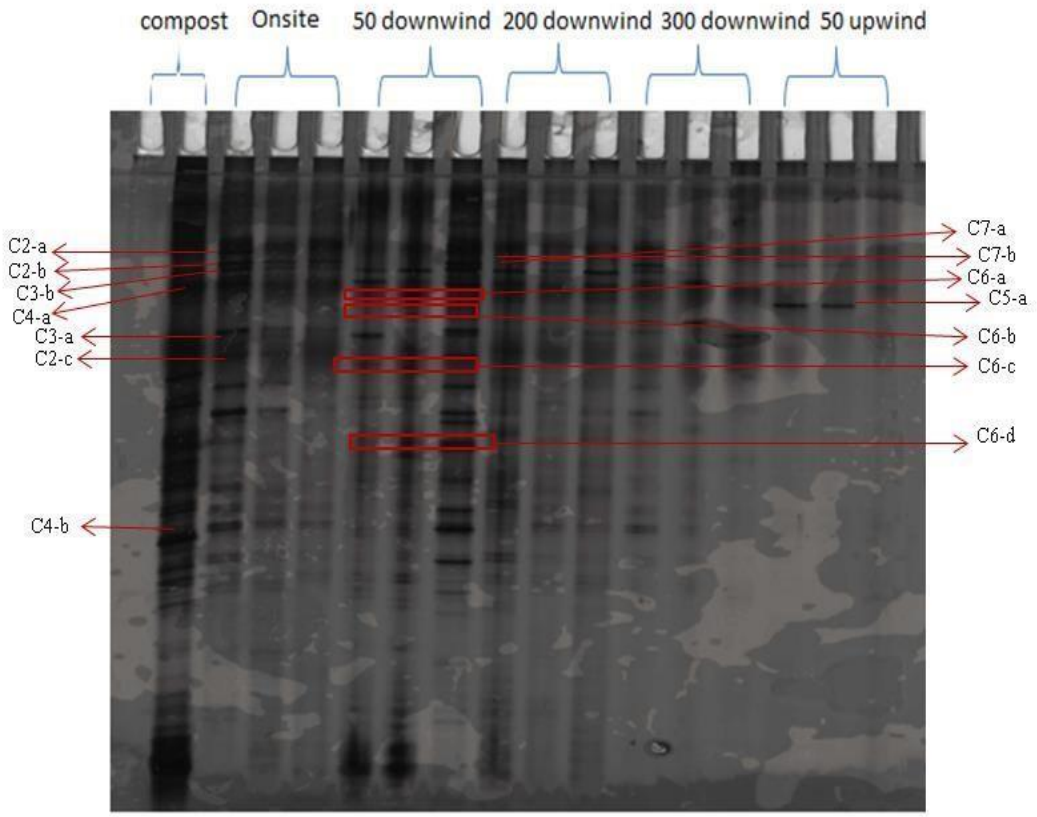

Figure 3.7 (a) DGGE profiles of bacterial 16S rDNA PCR amplicons of compost and bioaerosols from autumn season.

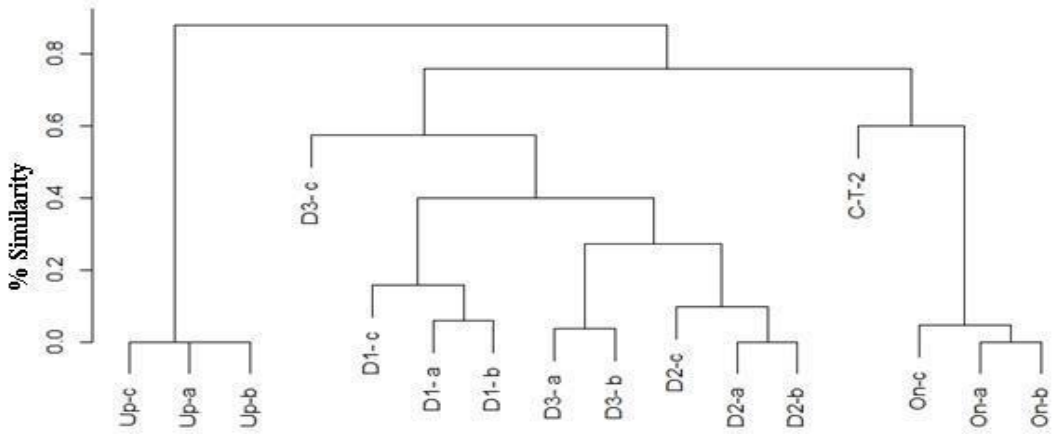

Figure 3.7 (b) Cluster dendrogram of the banding pattern in figure 3.7 (a) using a complete linkage setting based on jaccard similiraity index. C-T-2 = compost; Onsite = On- a, b, c; 50 m downwind = D1-a, b, c; 200 m downwind = D2- a, b, c; 300 m downwind = D3- a, b, c; 50 m upwind = UP-a, b, c.

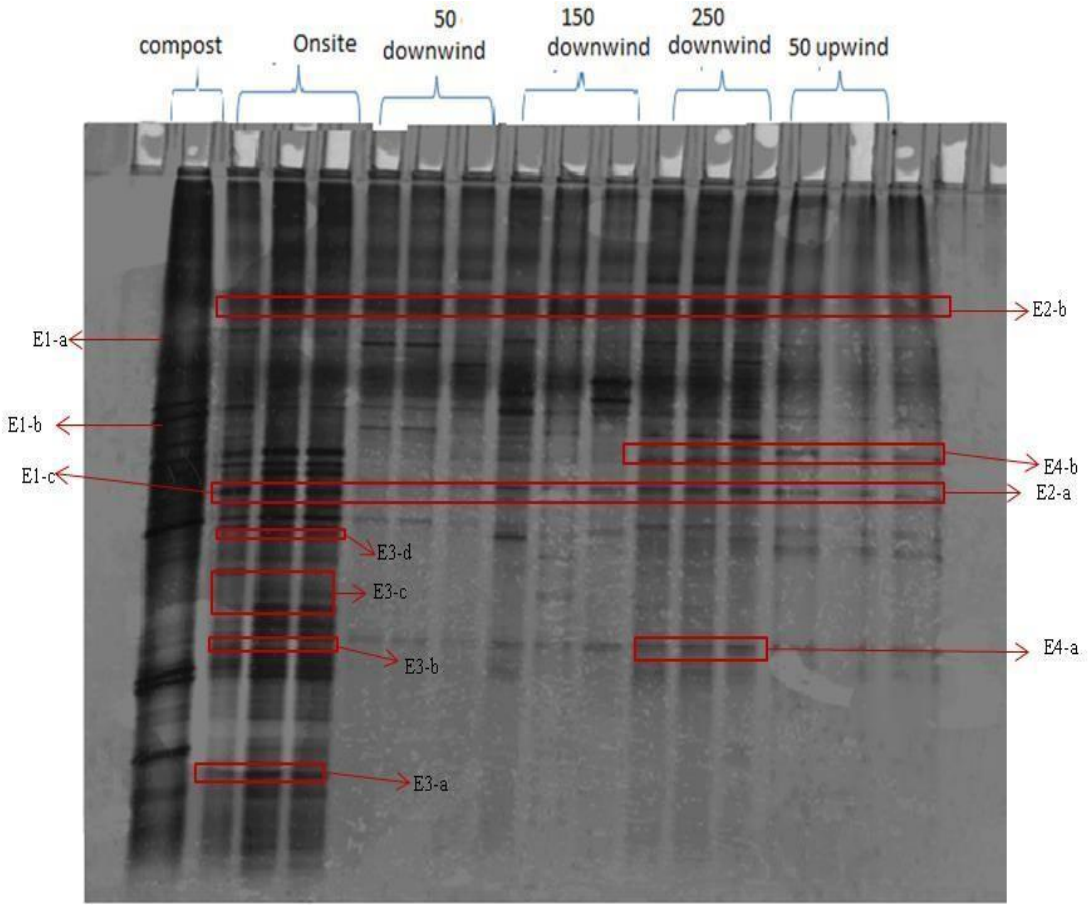

Figure 3.8 (a) DGGE profiles of bacterial 16S rDNA PCR amplicons of compost and bioaerosols from winter season.

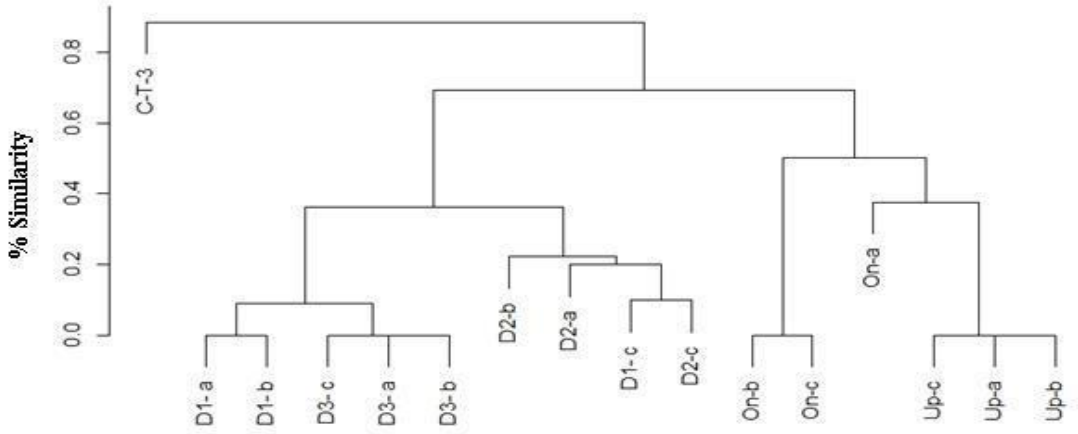

Figure 3.8 (b) Cluster dendrogram of the banding pattern in figure 3.8 (a) using a complete linkage setting based on jaccard similiraity index. C-T-3 = compost; Onsite = On- a, b, c; 50 m downwind = D1-a, b, c; 150 m downwind = D2- a, b, c; 250 m downwind = D3- a, b, c; 50 m upwind = UP-a, b, c.

3.2.4 Quantification of *Aspergillus fumigatus* over a seasonal cycle

The abundance of *Aspergillus fumigatus* in compost samples during different seasons were 7.3 x 10^6 ITS gene copies g^{-1} dry weight compost (summer), 5.5 x 10^7 ITS gene copies g^{-1} dry weight compost (autumn) and 8.2 x 10^6 ITS gene copies g^{-1} dry weight compost (winter) (Fig 3.9).

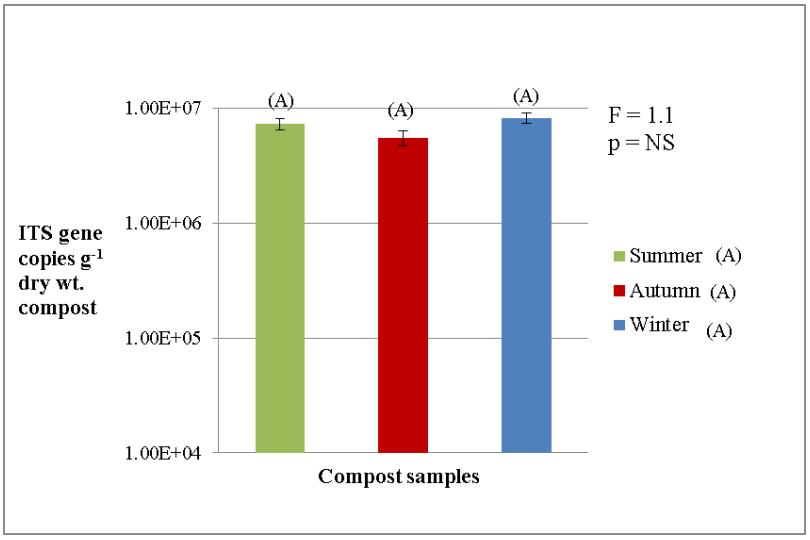

Figure 3.9 Abundance of *Aspergillus fumigatus* in compost in different seasons from Birch Airfield composting facility. Error bars represent standard error of mean (*n*=3). Similar letters in brackets after seasons or above bars indicate non- significant differences at 0.05 confidence interval.

3.2.5 Fungal community analysis in summer season.

In summer, DGGE analysis of fungal communities in the compost and bioaerosols indicated a diverse pattern of microbial community dynamics (Fig 3.10a) with characterization of 40% similarity observed between 200 m downwind samples and the compost sample (Fig 3.10b). Some of the band profiles were found in all samples with higher abundance as shown in "B1-a" depending on the brightness of the bands. Almost 70% similarity was observed between onsite and one of the downwind samples. "B4-a" represents the occurrence of the fingerprints in onsite and downwind samples; whereas "B3-a" and "B3-b" indicates the abundance of band profile onsite and 50 m downwind. "B5-a" specified abundance of compost species and "B7-a" indicated that compost fingerprints were found in

abundance onsite and downwind; however, some faint bands appeared in 50 m and 200 m downwind and represented the presence of fingerprints in "B6-a" and "B6-b & B6-c" (Fig 3.10a). Further, "B8-a" characterized the abundance of compost band profiles onsite and 50 m downwind; whereas, fingerprints decreased in intensity 200 m downwind as indicated by "B8-b". DGGE analysis of fungal communities in the compost and bioaerosols obtained during the autumn season indicated a fluctuating pattern of fungal communities' distribution among compost and bioaerosol samples (Fig 3.11a). Some of the band profiles were only found in the compost sample i.e. "D1-a-D1-f"; however, "D4-a- D4-b" depicted the occurrence of fingerprints only in the onsite samples. Further, D2-a- D2-b indicated presence of fingerprints in all the samples whereby brightness of the band indicated high abundance of species in the respective samples as compared with other fingerprints. Cluster analysis of the DGGE band distribution pattern indicated 40% similarity between fungal community dynamics at 250m downwind and compost samples whereas onsite samples showed 30% similarity with compost and 250 m downwind locations. Fingerprints distribution observed from the banding pattern in the "D3a- D3-b" indicated the presence of similar band profiles between the onsite, and downwind samples. However, dissimilar fungal community dynamics was observed at 300 m downwind and 50 m upwind of the site as shown by "D5-a" (Fig 3.11b).In winter, the DGGE profile of fungal rDNA amplified products revealed diverse fungal species distribution among compost, onsite and 50 m downwind of the site (Fig 3.12a). "F1-a & F1-b" represented the presence of similar band profiles in all of the samples. Cluster analysis of the DGGE banding pattern indicated that onsite fungal communities were 50% similar with the compost fungal population (Fig 3.12b). "F2-a- F2-b" also specified high abundance of fingerprints onsite and 50m downwind whereas "F3-a" represented the presence of similar fingerprints across all samples except 50 m upwind. Almost 40% of similarity was also observed between upwind and 50 m downwind fungal communities. Conversely, fungal population of 150 m downwind location was distantly related with the communities of others samples.

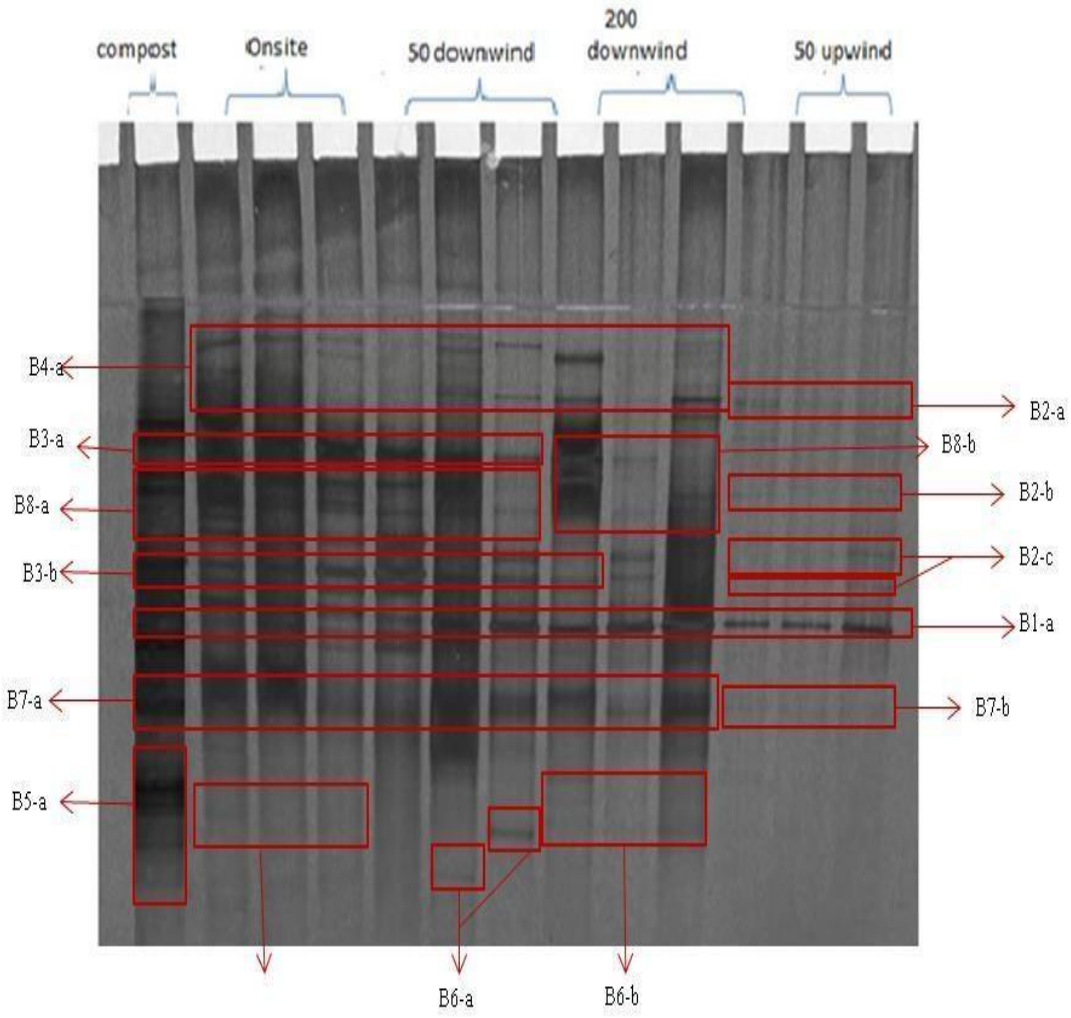

Figure 3.10 (a) DGGE profiles of fungal ribosomal DNA (ITS2 region) PCR amplicons of compost and bioaerosols from summer season.

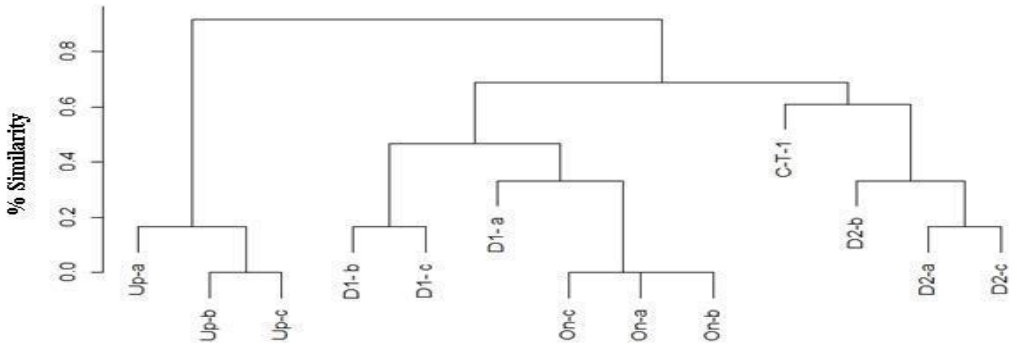

Figure 3.10 (b) Cluster dendrogram of the banding pattern in figure 3.10 (a) using a complete linkage setting based on jaccard similiraity index. C-T-1 = compost; Onsite = On - a, b, c; 50 m downwind = D1- a, b, c; 200 m downwind = D2- a, b, c; 50 m upwind = UP- a, b, c.

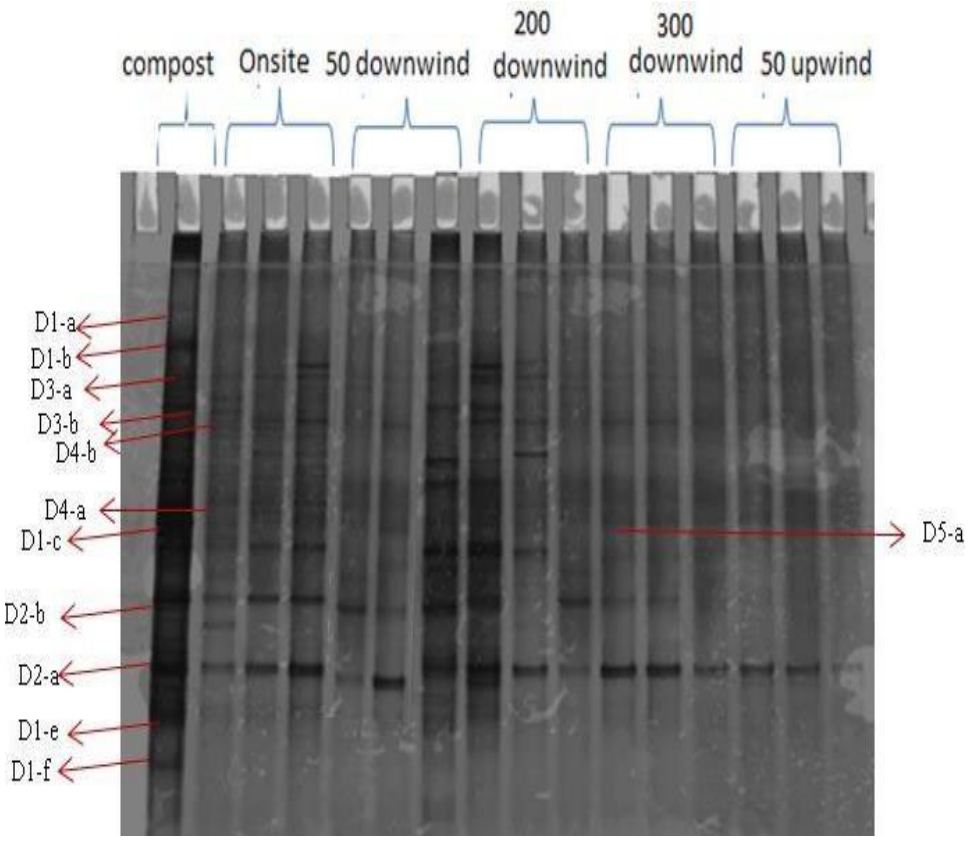

Figure 3.11 (a) DGGE profiles of fungal ribosomal DNA (ITS2 region) PCR amplicons of compost and bioaerosols from autumn season.

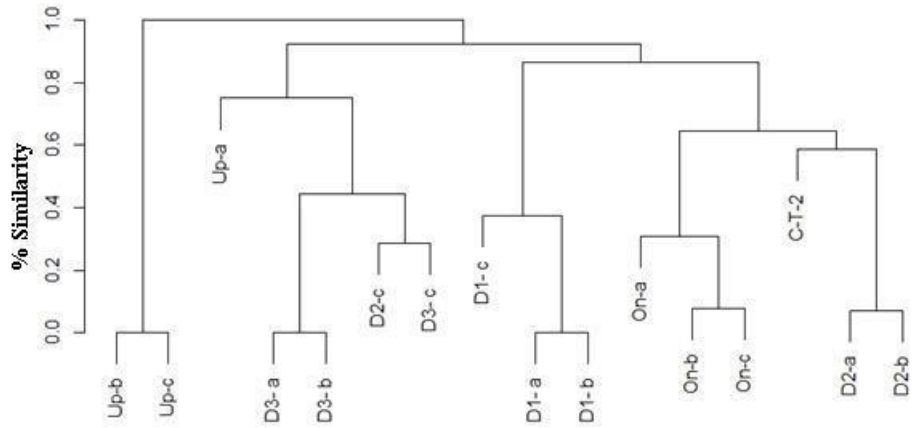

Figure 3.11 (b) Cluster dendrogram of the banding pattern in figure 3.11 (a) using a complete linkage setting based on jaccard similiraity index. C-T-2 = compost; Onsite = On- a, b, c; 50 m downwind = D1-a, b, c; 200 m downwind = D2- a, b, c; 300 m downwind = D3- a, b, c; 50 m upwind = UP-a, b, c.

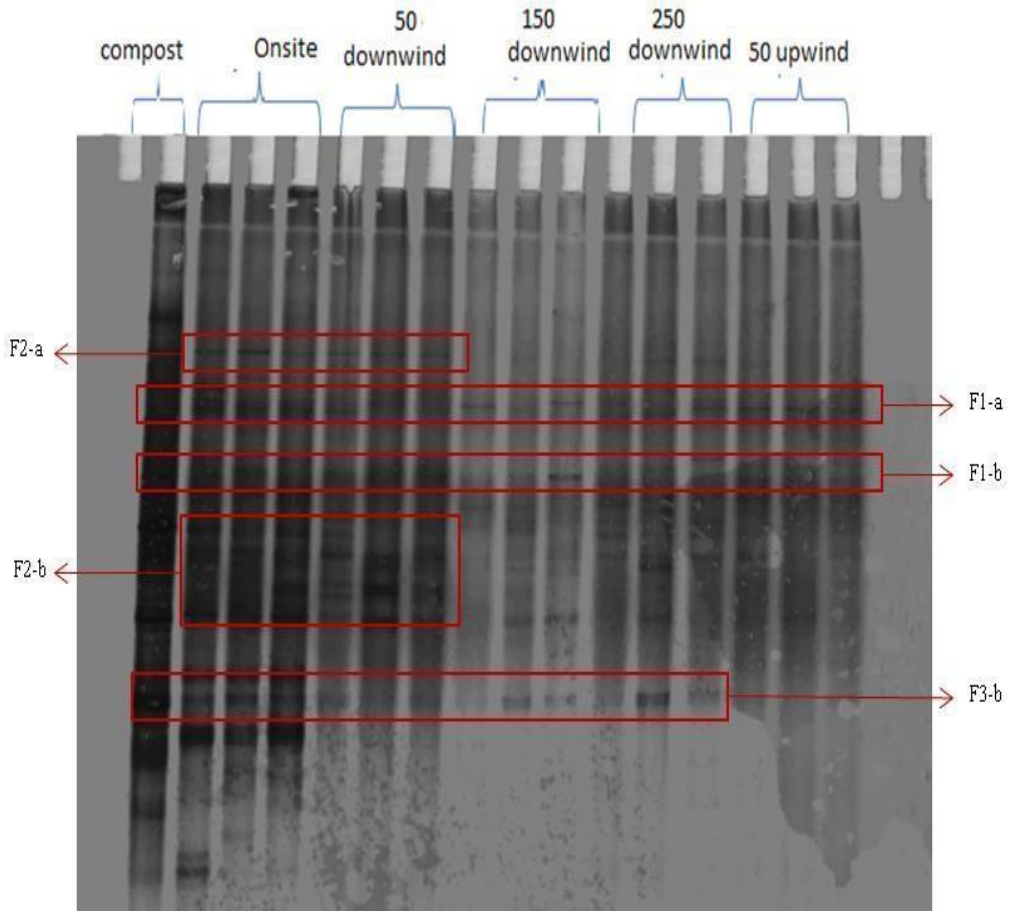

Figure 3.12 (a) DGGE profiles of fungal ribosomal DNA (ITS2 region) PCR amplicons of compost and bioaerosols from winter season.

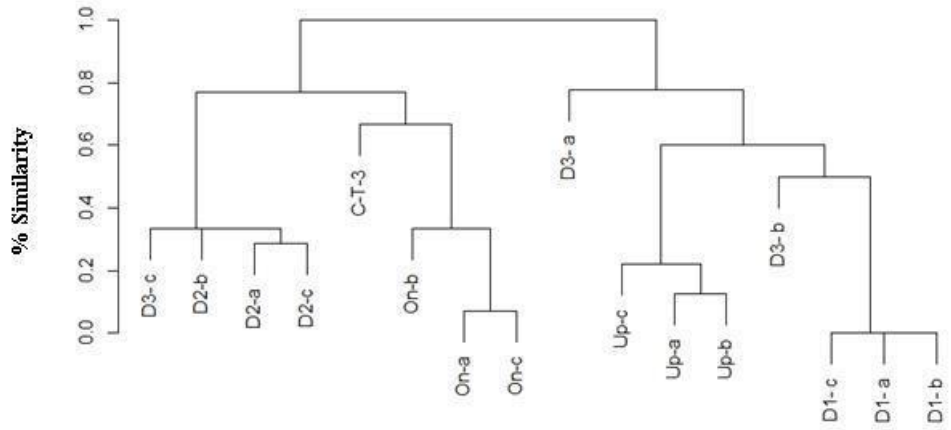

Figure 3.12 (b) Cluster dendrogram of the banding pattern in figure 3.12 (a) using a complete linkage setting based on jaccard similiraity index. C-T-3 = compost; Onsite = On- a, b, c; 50 m downwind = D1-a, b, c; 150 m downwind = D2- a, b, c; 250 m downwind = D3- a, b, c; 50 m upwind = UP-a, b, c.

3.2.6 Archaeal communities' analysis over a seasonal cycle

Archaeal communities were also investigated but were not detected in any of the samples throughout the seasonal cycle.

3.3 Discussion

The dispersal of bioaerosols from composting sites poses health associated risks to workers as well as residents living in proximity of the composting plant. Therefore, it is important to examine the composition and abundance of compost aerosols and factors such as site operations and meteorological settings that are considered to affect their composition and their dispersal. A limited amount of literature has been published to assess the impact of atmospheric conditions on the fate of microbial diversity of bioaerosols. Most of the published data regarding the microbial signature of bioaerosols are based on culture dependent analysis. Yet, a few studies have utilized molecular based techniques to examine the emissions of bioaerosols from composting plants (Pankhurst *et al.*, 2012; Le Goff *et al*. 2010; 2012; Schäfer *et al*., 2013). Further, there is also little understanding of microbial community composition and abundance mainly because of the health hazards associated with the exposure to bioaerosols from composting site (Le Goff *et al.*, 2010).

Results indicated that the abundance of total bacteria and *S. rectivirgula* were greatest onsite and a declining trend in abundance was observed with distance away from the source with lowest abundance upwind of the site. This is in agreement with Le Goff *et al*. (2011) and Williams *et al*. (2013) who reported a reduction in abundance of bioaerosols upwind of the site as compared with the downwind. The abundance of total bacteria did not reduce to upwind levels at 200 - 250 m downwind from the composting facility in summer and winter. Conversely, the abundance of bacteria in autumn at 250 m – 300 m were less than that of the upwind concentration. Previously, the Environment Agency (2007) has suggested site specific risk assessment if composting facilities have a working place or dwelling within 250 m of their boundaries after that concentrations are considered to reduce to background levels. However, in the published literature, there are disparities on the abundance of

bioaerosols beyond 250 m downwind of the site (Swan *et al.,* 2003; Recer *et al.,* 2001; Fischer *et al.,* 2008). Some studies suggested that the concentration could rise beyond a distance of 150 m downwind from the composting site (Swan *et al.,* 2003), whereas; other studies reported a decline in the abundance to background level beyond 500 m (Recer *et al.,* 2001; Fischer *et al.,* 2008).

Q-PCR was employed to quantify pathogenic microbial species i.e. *S. rectivirgula* and *A. fumigatus* in compost as well as bioaerosols emitting from the composting site. *Saccharopolyspora rectivirgula*, *Thermoactinomyces vulgaris* and *Aspergillus fumigatus* are generally regarded as common pathogenic species found at composting plants (Swan *et al.,* 2003). The abundance of *S. rectivirgula* across summer, winter and autumn followed the similar pattern as described for total bacteria. The abundance of *S. rectivirgula* recorded onsite ranged from 9.6×10^8 16S rRNA gene copies/m^3 (summer); 2.3×10^8 16S rRNA gene copies/m^3 (autumn) and 7.8×10^7 16S rRNA gene copies/m^3 (winter) respectively. Previously, Le Goff *et al.* (2011) quantified 2×10^6 to 4×10^7 copies of 16S rDNAm^{-3} onsite during site activity (turning). In another study, Schäfer *et al.* (2013) also documented onsite abundance of *S. rectivirgula* in bioaerosols between 1.24×10^2 cells m^{-3} to 1.5×10^7 cells m^{-3}. The abundance of *S. rectivirgula* was higher in summer and decreased with increasing distance; however, variations in abundance were observed at downwind distances (150d, 250d & 300d) between autumn and winter. This may possibly be because of differences in the comparison of downwind distances in different seasons due to access limitations across the composting site.Whilst *Aspergillus fumigatus* was quantified in compost samples, it was undetectable in biaoerosol samples. There are possible reasons for this pattern, for example the type of filter media utilized, sampling time and biases related to extraction procedure. The filter medium used in the research was polycarbonate filter and it has been stated that polycarbonate filters hold the lowest collection efficiencies (22 -49%) as compared with the efficiencies (<93%) of gelatin and polytetrafluoroethylene filters (Burton *et al.,* 2007). The collection time (30 min) and low flow rate (2l/min) may also effect the collection of *A. fumigatus* spores. Also, the biases during DNA extraction of environmental samples may be

the possible reasons in this regard (Einen *et al.,* 2008; Schafer *et al.,* 2011).Furthermore, it can be possible that compost piles during the time of sampling were not at the maturation phase which could have resulted in non-detection of *Aspergillus fumigatus* in the compost. It has been suggested that during maturation stage fungal population increase significantly to degrade chitin, lignin and cellulose utilizing extracellular enzymes into simpler products and curing piles (decline in temperature) provide appropriate settings for fungus to grow and degrade material as these prefer cooler temperature (Millner *et al.,* 1977).

It has been reported in the literature that site specific activities, natural and physical features of the site and the surroundings and meteorological settings, mainly temperature and wind speed affect the microbial community composition and abundance of the bioaerosols (Swan *et al.,* 2003; Pankhurst *et al.,* 2011; Wery, 2014). Concerning the effect of seasonal parameters on the fate (composition & abundance) of bioaerosols, it was anticipated that the higher temperature would result in the increased dispersion of bioaerosols as compared with lower temperatures.

Meteorological variables such as, temperature, humidity and ultra violet radiation are suggested to influence the dispersal and initial release of bioaerosols (Jones and Harrison, 2004). It was reported that temperature and relative humidity holds a positive and negative relationship with the ambient bioaerosols respectively (Grinn-Gofroń and Strzelczak, 2008). Correspondingly, results in the present study indicated that the abundances attained during seasonal sampling regimes revealed maximum emission of total bacteria and *S. rectivirgula* during the summer as compared with autumn and winter. This increase in abundance is in agreement with previous published literature (Recer *et al.,* 2001, Schlosser *et al.,* 2009), where higher microbial diversity was suggested during the warmer season. It has been reported that warm temperatures enhance bacterial and fungal growth resulting in higher levels of these bioaerosols in summer and spring (Rathnayake *et al.,* 2016). The higher emission in summer season would be due to low humidity in the atmosphere as high humid air hinders the dispersal of aerosols (Lighthart & Mohr, 1987). Further, high temperature

result in the drying of compost that easily becomes airborne due to onsite activities. Moreover, the impact of seasonal variations on the microbial abundance was not consistent in autumn and winter as compared with summer. Regarding autumn and winter, variations in bioaerosol abundances were observed at different locations across composting site, whereby total bacteria abundances during autumn were higher onsite and 50 meters downwind and lower at 150 meters and 250 meters as compared with winter. Likewise, during winter, the total bacteria abundance observed at 150 meters (instead of reduction) was higher than 50 meters downwind of the composting site. This variation in bioaerosol abundance may be due to the onsite activity i.e. shredding, carrying out during sample collection at 50 meters downwind as compared with 150 meters downwind whereby turning activity was being carried out. It was reported that turning of the compost pile generates higher abundance of actinomycetes (Lacey, 1997); whereas, shredding leads to lower emissions of *A. fumigatus* and actinomycetes (Schlosser *et al.,* 2009). However, screening of the compost results in the increased dispersal of gram- negative bacteria, actinomycetes and endotoxins.

The treatment of vast amount of waste materials involves more activities that lead to the increased dispersal of microorganisms from the site. It has been reported by Steger *et al*. (2005) that handling of the composting process with different physical features influences the microbial community dynamics of the compost and results in different microbial populations. Adams and Frostick (2009) reported that the feedstock composition is likely to effect the microbial community composition of the composting process. Hence, the difference in the composition would affect the microbial community dynamics of the bioaerosols.

It has been suggested that an increase of 2 – 3 orders of magnitude of biaoerosol dispersal can result from activities taking place on the composting site (Sykes *et al.,* 2007; Taha *et al.,* 2006). Further, total bacteria abundance was similar between 250 and 300 m downwind in autumn and a higher abundance was achieved at 150 m downwind as compared with 50 m downwind of the site. Previously, it has been reported that an increase in the abundance of

bioaerosols above 3 orders of magnitude was observed due to site activities (Le Goff *et al.*, 2011). Similarly, wind speed (4.6 m/s) during the sample collection at 150 meters downwind was also higher than at 50 meters downwind of the site (table 3.1 & fig 3.1). Higher wind speed is considered to influence the emissions of compost aerosols (Jones and Harrison, 2004; Swan *et al*, 2003). It has been described that an increase in ambient wind speed would result in the rapid dispersion of bioaerosols (Oke, 1987). Previously published studies have reported a range of wind speeds from 0.1 to 4 m/s while assessing the dispersion of bioaerosols across composting sites (Taha *et al.*, 2004; Taha *et al.*, 2006; Le Goff *et al.*, 2011; Pankhurst *et al.*, 2012). Average wind speed recorded during summer (5.1 m/s) was higher than other seasons; conversely, lowest average wind speed was recorded during winter i.e 3.9 m/s (table 3.1& fig 3.1). Further, wind speed recorded in each respective season also varied during different sampling periods of a particular sampling day. However, a negative correlation was indicated between the wind speed and bioaerosol abundance only in the summer as compared with winter and autumn.

Moreover, DGGE fingerprint analysis of bacterial communities during summer, autumn and winter season presented a difference between microbial composition and abundance at different locations during different seasons. This has also been stated by Pankhurst *et al.* (2012), where microbial community analysis (DGGE) of the bioaerosols from composting sites indicated a significant difference between microbial community structure of compost and airborne communities of onsite, downwind and upwind samples. In the summer, DGGE analysis indicated >20% similarity between compost and onsite bacterial communities; whereas, the microbial community structure of compost and onsite aerosol in autumn and winter was 40% and 10% similar respectively.

Nonetheless, these dissimilarities in microbial signature of bioaerosols may be due to the presence of the main source (compost) as well as onsite operational activities that result in the dispersion of communities present in the source in air. This is evidenced from Le Goff *et al.*, (2010), where airborne microbial species were also traced back with the compost samples. Apart from disparities in the community structure of compost and airborne

bacterial communities, significant variations in bioaerosol community composition were recorded across different seasons. Although almost 10% similarity was observed between onsite and upwind microbial fingerprints during summer and autumn; yet <50% similarity was observed during winter. Likewise, higher similarity (>40%) of bacterial communities was obtained between onsite and 50m -200m downwind in summer as compared with autumn and winter i.e. >30% and <30% respectively. Again, these variations in bacterial communities' composition have been suggested to be dependent on atmospheric patterns, composition of the feedstock and onsite activities during the corresponding sampling period (Swan *et al.,* 2003; Le Goff *et al.,* 2010).

Furthermore, the variations between the microbial community dynamics of bioaerosols emitted during different seasons can be associated with the availability of the substrates found in the composting environment. Studies have suggested that physiochemical characteristics of the substrate influence the metabolic abilities of the microbial communities and the process of organic decomposition as well as stabilizing the organic matter during the composting process (Francou *et al.,* 2005; Lyons *et al.,* 2006; Zhang *et al.,* 2016). The utilization of the amount of substrates present in the feedstock i.e. sugar, cellulose, lignin and hemicellulose by microorganisms determines the population and abundance of microbial communities; with one group dominating others at times, hence leading to the succession of microbial communities during the composting process (Begon *et al.,* 1996). For instance, mesophilic bacteria dominate the beginning of the process, followed by actinomycetes, thermophilic bacteria and fungi in thermic phase and eventually lead to measophilic bacteria and actinomycetes in the curing phase of the process (Fogarty & Tuovinen, 1991; Miller, 1996).

In order to gain insight of microbial diversity of compost associated bioaerosols, a few researches have utilized DNA sequencing technology (Adan *et al.,* 2009; Le Goff *et al.,* 2010; Pankhurst *et al.,* 2012). In a study Adan *et al.* (2009) reported Firmicutes (46% of sequences), Actinobacteria (28% of sequences) and Alphaproteobacteria (10% of sequences) as most abundant phylum. The most abundant bacterial phylotypes obtained

were *Thermobifida fusca* (phylum actinobacteria) and *Thermoactinomyces thalpophilus* (phylum firmicutes). However, the most abundant fungal phylotypes were basidiomycetes (59% of sequences), 9% of sequences were closely related to *Aspergillus* species among ascomycetes (Adan *et al.*, 2009).

In another study Le Goff *et al.* (2010) showed that dominant bacterial phyla belonged to *Firmicutes* (95 phylotypes, 328 sequences) and *Actinobacteria* (63 phylotypes, 263 sequences). Among bacterial phylotypes, only *Saccharopolyspora rectivirgula* were present at all 5 composting sites. Concerning fungal diversity, ascomycota was found as the dominant fungal phyla with 67% dominance in 21 fungal phylotypes (Le Goff *et al.*, 2010). Pankhurst *et al.* (2012), whilst utilizing the pyrosequencing technology to sequence the bacterial communities present in the bioaerosols emitted from composting sites showed the abundance of *Firmicutes, Actinobacteria, Alpha- and Gamma- proteobacteria* in all samples whereas *Actinobacteria* was absent in downwind samples. There was a high level of dominance *of Pseudomonas* in downwind samples. Thirty nine pathogenic phyla were identified in the bioaerosols and out of those 10 phyla were recognised as human pathogens (Pankhurst *et al.*, 2012).

Bioaerosol emissions decreased with increasing distances and the majority of the microbial population followed this configuration; however it was also assumed that the fungal bioaerosol signature would disperse with longer distances across composting sites as compared to bacteria. DGGE profiles of fungal rDNA confirmed a higher occurrence of fungal species in the furthest downwind locations (250 – 300 m). Cluster analysis of fungal fingerprints indicated >60% similarity between onsite and 200 meters downwind in airborne communities during the summer which is higher than bacterial airborne communities. Similarly, during autumn and winter, 40% and 20% similarity was recorded between fungal fingerprints onsite and 250 meters downwind. This pattern of increased dispersal may be because of the ease with which fungal spores become airborne during composting (Fletcher *et al.*, 2008). Also, the increased dispersion of fungal aerosols relative to bacteria depends on the drier conditions of the compost as it has been suggested that drier compost favours

the dispersal of spore forming microbes. This could be a possible reason for increased dispersal of fungal communities downwind of the site (Le Goff *et al*., 2010).

Furthermore, in this research, sequence analysis (MiSeq) on the collected samples will be undertaken at later date to gain in-depth understanding of the microbial community composition of the bioaerosols. There has been a limited knowledge about the community composition and abundance of compost associated aerosols due to dependency on culture based analysis. Yet few researchers have utilized sequencing techniques to investigate the microbial signature of bioaerosols from composting sites (Adan *et al.,* 2009; Le Goff *et al.,* 2010; Pankhurst *et al.,* 2012). The application of sequencing technology would enhance the knowledge of microbial ecology of compost aerosols and contribute significantly in the characterization of emissions to delve the impact of daily and seasonal variations and other parameters i.e. feedstock composition and site activities.

3.4 Conclusions

Findings achieved in this research illustrate higher emissions of bioaerosols from source at composting sites which tend to decrease with increasing distance from the site. Maximum abundance of bioaerosols was obtained in summer. However, microbial community analysis showed significant differences between the microbial community dynamics and abundance across the composting site. Likewise, the presence of higher amounts of fungal communities at downwind locations indicated long distance dispersal of fungal spores relative to bacteria.

Chapter 4 An investigation of microbial diversity of bioaerosol emissions from composting facilities in Lahore, Pakistan

Introduction

Approximately, 200 million tonnes of solid waste is generated per annum in Pakistan and, in particular, Lahore produces more than 20 million tonnes of municipal solid waste annually (5300 tonnes/day), out of which 64.8 % is biodegradable waste (ISTAC, 2012; Masood *et al.*, 2014). The significant rise in the amount of waste generation in Lahore is mainly due to rapid urbanization and the city's expansion. However, due to a lack of awareness about waste recycling techniques, an enormous amount of generated waste is still dumped on a large scale at un-engineered open sites.Composting has been emerging as a contemporary waste management approach in major cities (Lahore & Karachi) of Pakistan and currently two composting facilities are operating in Lahore. Yet, as a new waste recycling strategy, there is deficiency of information about the health hazards associated with the dispersal of bioaerosols onsite and off-site of composting facilities, and to date, no published literature has been reported to characterize the emission of bioaerosols from waste management sites in Pakistan i.e. composting, land-fills and open dumping sites. Currently, three waste dumping sites are situated in Lahore where illegal waste burning is commonly practiced.

The aim of the research was to quantify and characterise the microbial communities found in the emissions of compost aerosols from composting sites in Pakistan. It is hypothesised that relative to bioaerosol release from the UK facility in summer there will be a higher microbial abundance in bioaerosols emitted from the Pakistan's composting sites due to the influence of higher temperatures and low humidity. This is the first study to assess the exposure to bioaerosols from composting sites in Pakistan.

Specifically, this chapter investigated how meteorology affects the dispersal and fate of bioaerosols in Pakistan compared to that in the UK. A small scale study was also undertaken in a village in Pakistan to assess the microbial diversity of bioaerosols emitted from a manure

heap (anaerobic decomposition). The findings of this study will help in the mitigation of health hazards faced by the workers and local residents. The findings will not only assist in an evaluation of exposure to bioaerosols emissions at compositing sites but also greatly contribute towards the knowledge on the impact of different meteorological/climatic conditions on microbial diversity.

Results

A summary of sample types & ID, onsite activities, locations and meteorological data obtained during the seasonal sampling regime at the UK composting site is given in Table 4.1 and Figure 4.1.

Table 4.1 Description of bioaerosol sampling operation during Pakistan sampling campaign.

Sites	Sample type	Sample ID	Onsite activities	location	Wind speed (m/s)	Humidity (%)	Temperature (^{o}C)
Waste Buster	Air	Up-a, b, c	screening,	50m upwind	0.4	43.7	35.2
Waste Buster	Air	On-a, b, c	screening, Turning	onsite	0.2	40	37.3
Waste Buster	Air	D1- a, b, c	screening, shredding	50m downwind	0.5	40.7	37.9
Waste Buster	Air	D2-a, b, c	screening shredding	250m downwind	0.2	39.9	38.3
Waste Buster	Compost	WB-C	-	-	-	-	-
Lahore compost	Air	Up-a, b, c	-	50m upwind	0.2	40.3	38.1
Lahore compost	Air	On-a, b, c	shredding	onsite	0.2	41.6	37.8
Lahore compost	Air	D1- a, b, c	-	50m downwind	0.3	44.7	34.7
Lahore compost	Compost	LH-C	-	-	-	-	-
Village	Air	A, B, C, D, E, F	-	5-10 m nearby	1.2	42.7	32.2
Village	Compost	Vill -C	-	-	-	-	-

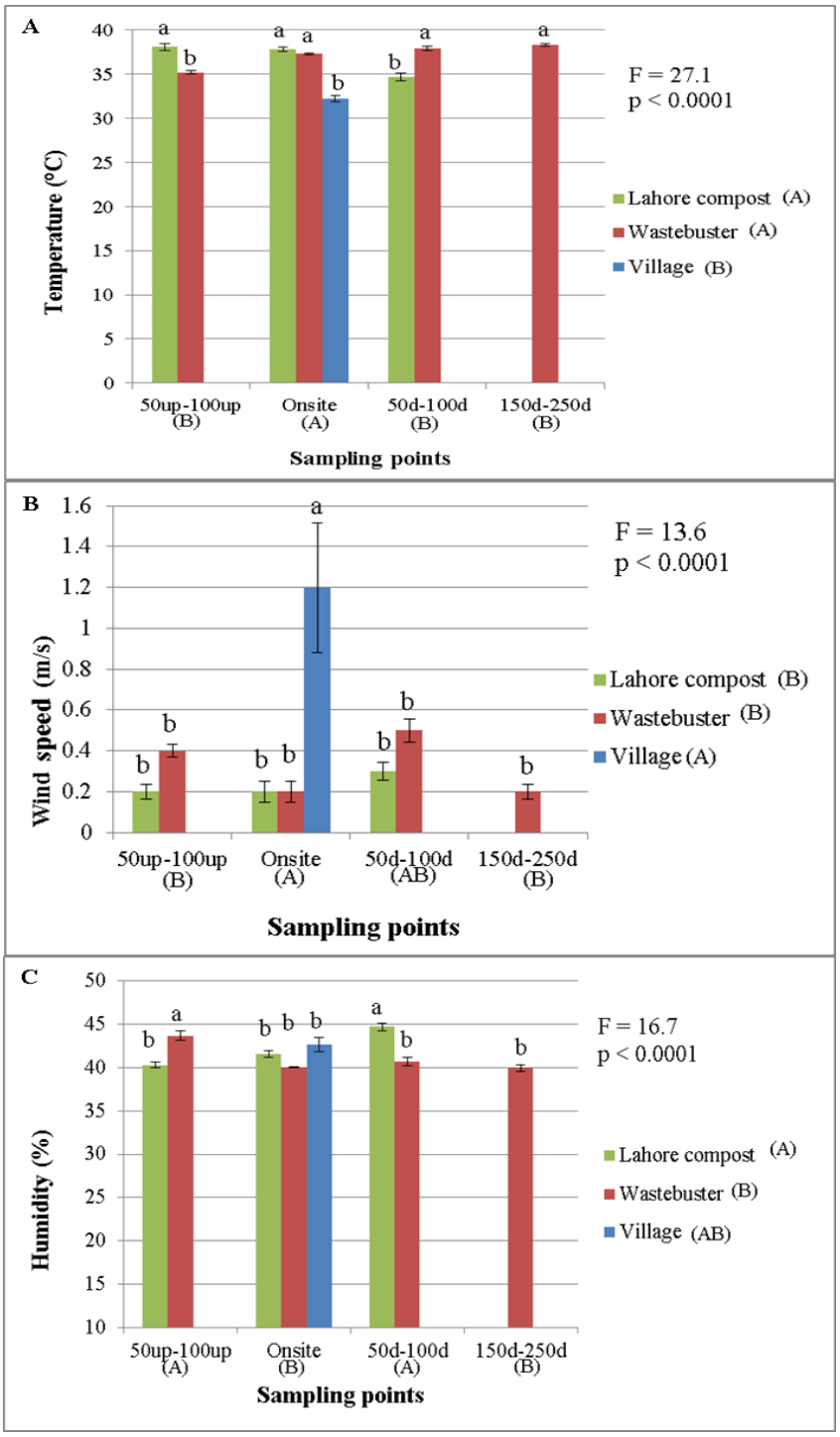

Figure 4.1 Temperature (A), wind speed (B) and humidity (C) recorded during Pakistan sampling campaign. 50up – 100up = 50m – 100 m upwind; 50d – 100d = 50m – 100 m downwind; 150d – 250d = 150d – 250 m downwind of the composting sites. Error bars represent standard error of mean (*n*=6). Significant differences are shown by ANOVA and Tukey's HSD. Different letters in brackets after composting sites, sampling points or above bars indicate significant differences at 0.05 confidence interval.

4.2.1 Quantification of total bacteria in the composting facilities and manure heap.

The abundance of total bacteria recorded at the Waste Buster composting site indicated an increase of one order of magnitude between 50 m upwind and onsite; whereas a decrease of one order of magnitude was observed between onsite and downwind samples (Table 4.2) (Fig 4.2). At the Lahore composting site, an increase of two orders of magnitude was observed onsite as compared to 50 m upwind and 50 m downwind of the site (Table 4.2) (Fig 4.2). The abundance of total bacteria recorded in Waste Buster, Lahore compost and village compost samples were 1.57 x 10^{10} 16S rRNA gene copies g^{-1}; 7.06 x 10^{9} 16S rRNA gene copies g^{-1} and 1.38 x 10^{10} 16S rRNA gene copies g^{-1} respectively (Fig 4.3).

Table 4.2 Quantification of total bacteria in bioaerosols emitted from Pakistan composting facilities and village manure heap.

Sampling points	Lahore compost	Wastebuster	Village
50up-100up	3.2×10^7	3.3×10^7	
Onsite	9.1×10^9	5.1×10^8	3.3×10^8
50d-100d	3.2×10^7	8.4×10^7	
150d-250d		9.6×10^7	

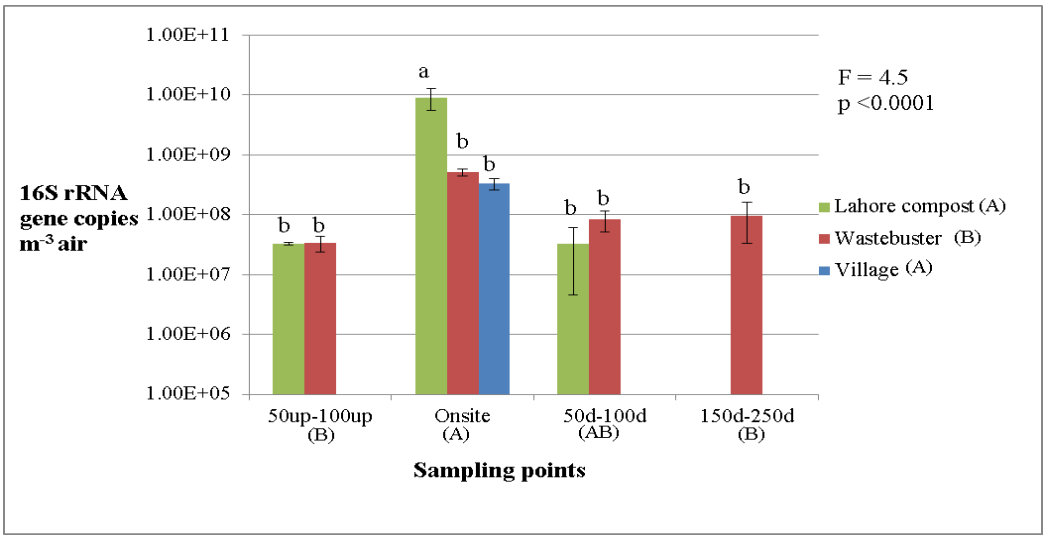

Figure 4.2 Abundance of bacteria in bioaerosols emitted from Pakistan composting facilities and village manure heap. 50up – 100up = 50m – 100 m upwind; 50d – 100d = 50m – 100 m downwind; 150d – 250d = 150d – 250 m downwind of the composting sites. Error bars represent standard error of mean (*n*=3). Significant differences are shown by ANOVA and Tukey's HSD. Different letters in brackets after composting sites, sampling points or above bars indicate significant differences at 0.05 confidence interval.

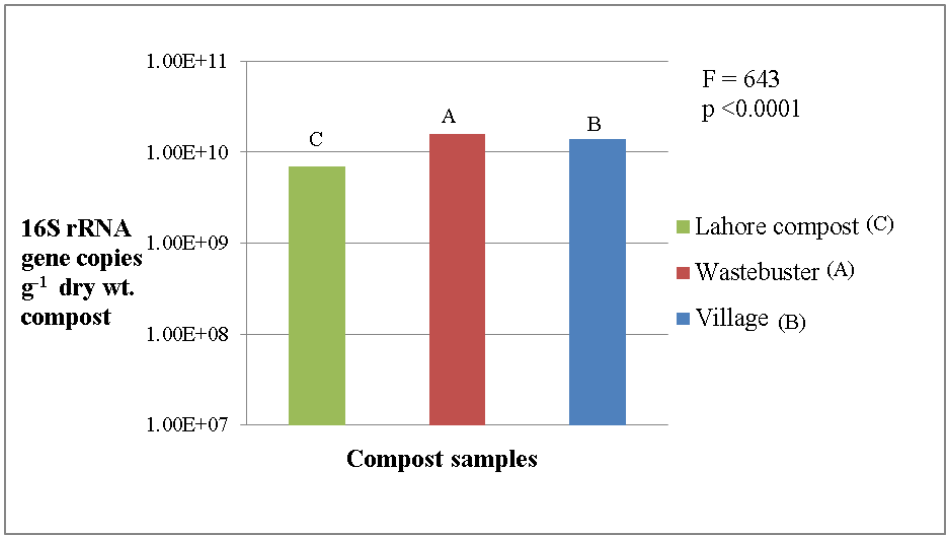

Figure 4.3 Abundance of bacteria in compost from Pakistan composting facilities and village manure heap. Significant differences are shown by ANOVA and Tukey's HSD. Different letters in brackets after composting sites, or above bars indicate significant differences at 0.05 confidence interval.

4.2.2 Quantification of *Saccharopolyspora rectivirgula* in the composting facilities and manure heap.

At the Waste Buster composting site, abundances of *Saccharopolyspora rectivirgula* indicated an increase of one order of magnitude between 50 m upwind and onsite and between 50 m upwind and downwind samples (Table 4.3) (Fig 4.4). At Lahore compost site, abundances of *Saccharopolyspora rectivirgula* was maximum at onsite and minimum 50 m upwind of the site (Table 4.3). In the village, the abundance obtained for *Saccharopolyspora rectivirgula* was 6.1 x 10^7 16S rRNA gene copies/m^3 (Fig 4.4). The abundance of *Saccharopolyspora rectivirgula* recorded at Waste Buster, Lahore compost and village compost samples were 7.1 x 10^8 16S rRNA gene copies g^{-1}, 6.65 x 10^7 16S rRNA gene copies g^{-1}, 3.1 x 10^8 16S rRNA gene copies g^{-1} respectively (Fig 4.4a).

Table 4.3 Quantification of *S. rectivirgula* from Pakistan composting facilities and village manure heap

	Lahore compost	**Wastebuster**	**Village**
50up-100up	3.1×10^6	3.3×10^6	
Onsite	9.7×10^6	2.7×10^7	7.05×10^6
50d-100d	8.2×10^6	2.2×10^7	
150d-250d		1.6×10^7	

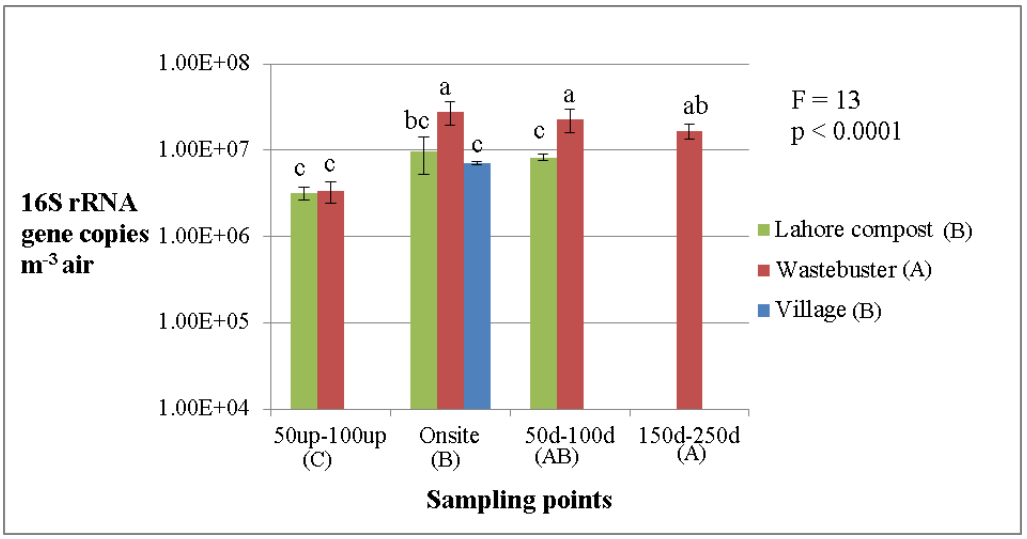

Figure 4.4 Abundance of *S. rectivirgula* in bioaerosols emitted from Pakistan composting facilities and village manure heap. 50up – 100up = 50m – 100 m upwind; 50d – 100d = 50m – 100 m downwind; 150d – 250d = 150d – 250 m downwind of the composting sites. Error bars represent standard error of mean (*n*=3). Significant differences are shown by ANOVA and Tukey's HSD. Different letters in brackets after composting sites, sampling points or above bars indicate significant differences at 0.05 confidence interval.

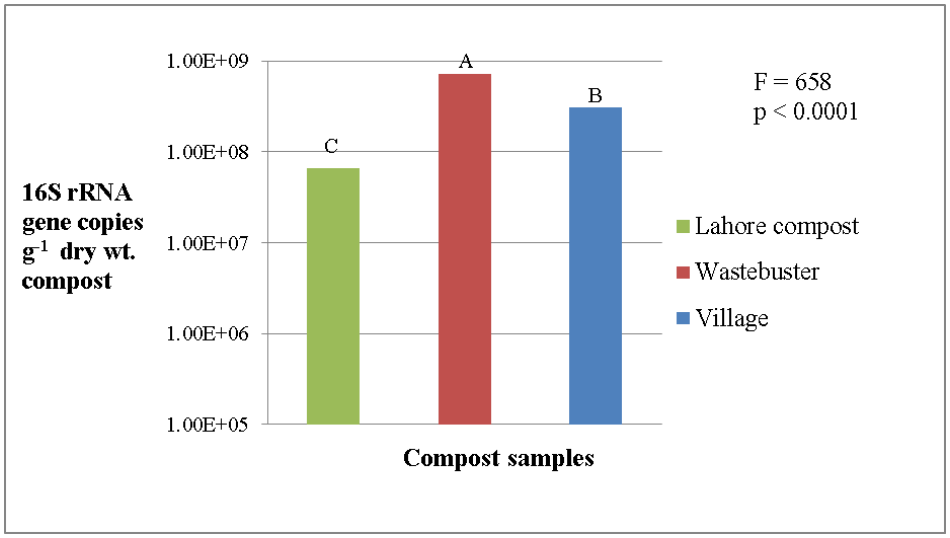

Figure 4.4a Abundance of *S. rectivirgula* in compost from Pakistan composting facilities and village manure heap. Significant differences are shown by ANOVA and Tukey's HSD. Different letters above bars indicate significant differences at 0.05 confidence interval.

4.2.3 Quantification of *Aspergillus fumigatus* in the composting facilities and manure heap.

Aspergillus fumigatus was not detected in the bioaerosol samples, whereas its concentration in the Waste Buster, Lahore compost and village compost samples were 2.34×10^5 ITS gene copies g^{-1}; 9.08×10^7 ITS gene copies $g^{-1}$and 4.43×10^6 ITS gene copies g^{-1} respectively (Fig 4.5).

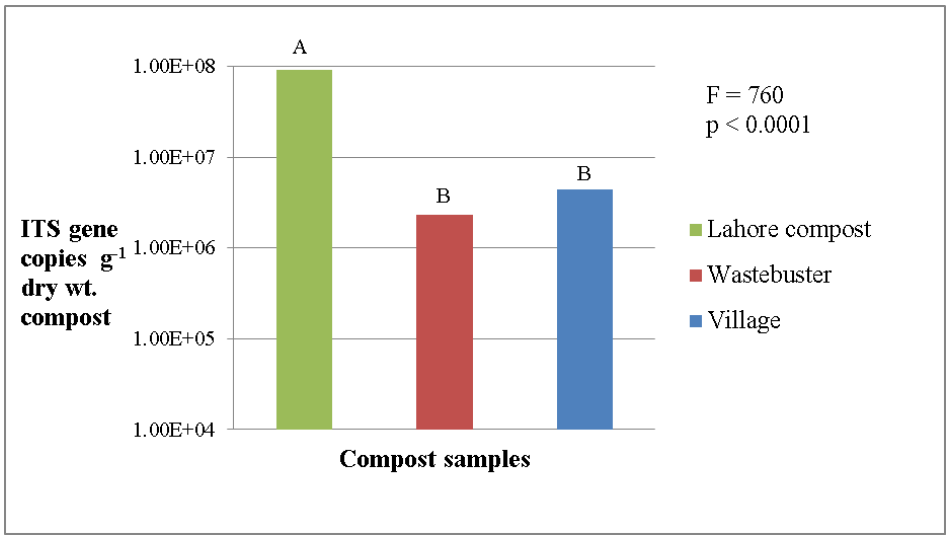

Figure 4.5 Abundance of *Aspergillus fumigatus* in compost from Pakistan's composting facilities and village manure heap. Significant differences are shown by ANOVA and Tukey's HSD. Different letters above bars indicate significant differences at 0.05 confidence interval.

4.2.4 Bacterial community analysis in the composting facilities and manure heap. The DGGE banding pattern of bacterial 16S rDNA PCR amplified products from the Waste Buster composting facility indicated the existence of more or less similar microbial communities across all the samples as shown in Figure 4.6a"G1-a – G1-d". However, "G2-a & G2-b" indicated diverse fingerprints in bioaerosols as compared with the compost (Fig 4.6a). Hierarchal cluster analysis of the banding pattern indicated approximately 80% similarity of bacterial communities between compost, onsite and 50 meters downwind as compared with upwind and 250 downwind samples. However, communities found 250 meters downwind and 50 meters upwind shared almost 50% similarity with each other (Fig 4.6b). DGGE profiles of bacterial 16S rDNA PCR amplicons obtained from the Lahore compost facility specified diverse microbial communities of compost samples which indicated a resemblance to those of the onsite population. The banding pattern showed that a similar fingerprint profile was recorded in all the samples as indicated in Figure 4.7a "I1-a-I1-d". However, "I2-a – I2-b" specified a high abundance of microbial flora in onsite samples as compared with the rest of the samples (Fig 4.7a). Cluster analysis indicated 60% similarity between compost and onsite samples, whereas 20% similarity was observed between compost and the cluster of downwind and upwind samples. However, the banding pattern indicated almost 40% similarity between downwind and upwind samples (Fig 4.7b). A higher distribution of bacterial species was observed in compost and bioaerosols from the manure heap. The banding pattern indicated the presence of similar profiles in all the samples as indicated by Figure 4.8a "K1-a – K1-c". Some of fingerprints were abundant in aerosol samples as compared to the compost i.e. "K2-a – K2-b" (Fig 4.8a). Cluster analysis of the banding pattern represented almost 50% similarity with the aerosol samples. Considerably lower biomass was observed in sample "C" of bioaerosol as compared with other samples, however, occurrence of faint bands, showed almost 30% similarity with other samples (Fig 4.8b).

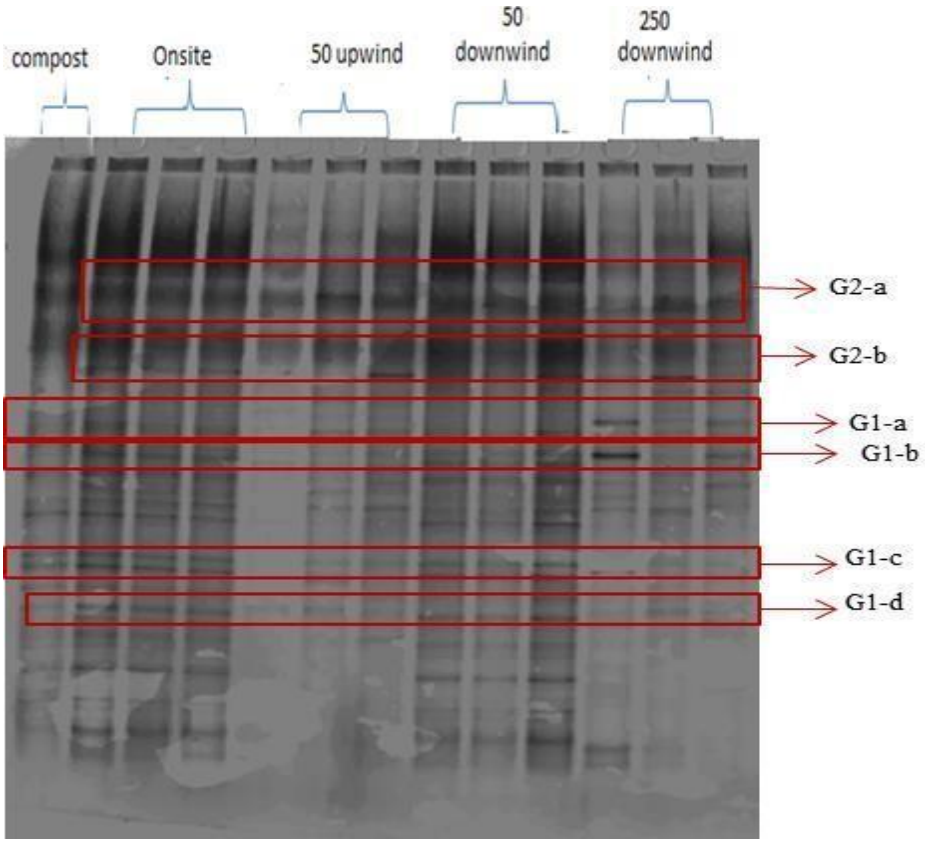

Figure 4.6 (a) DGGE profiles of bacterial 16S rDNA PCR amplicons of compost and bioaerosols from Waste Buster composting facility, Lahore Pakistan.

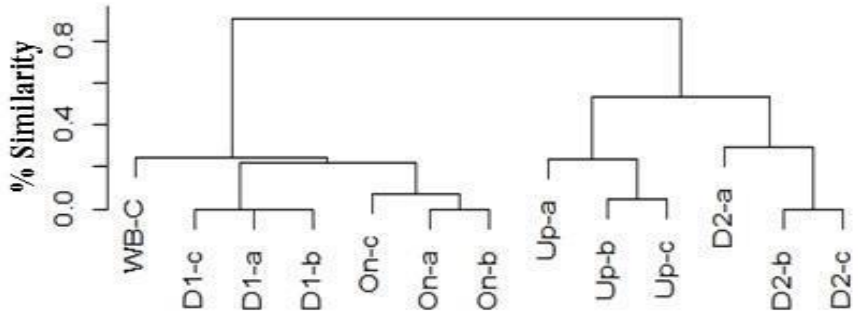

Figure 4.6 (b) Cluster dendrogram of the banding pattern in figure 4.6 (a) using a complete linkage (Jaccard cofficient). WB-C = compost; Onsite = On - a, b, c; 50 meters downwind = D1- a, b, c; 250 meters downwind = D2- a, b, c; 50 meters upwind = UP- a, b, c.

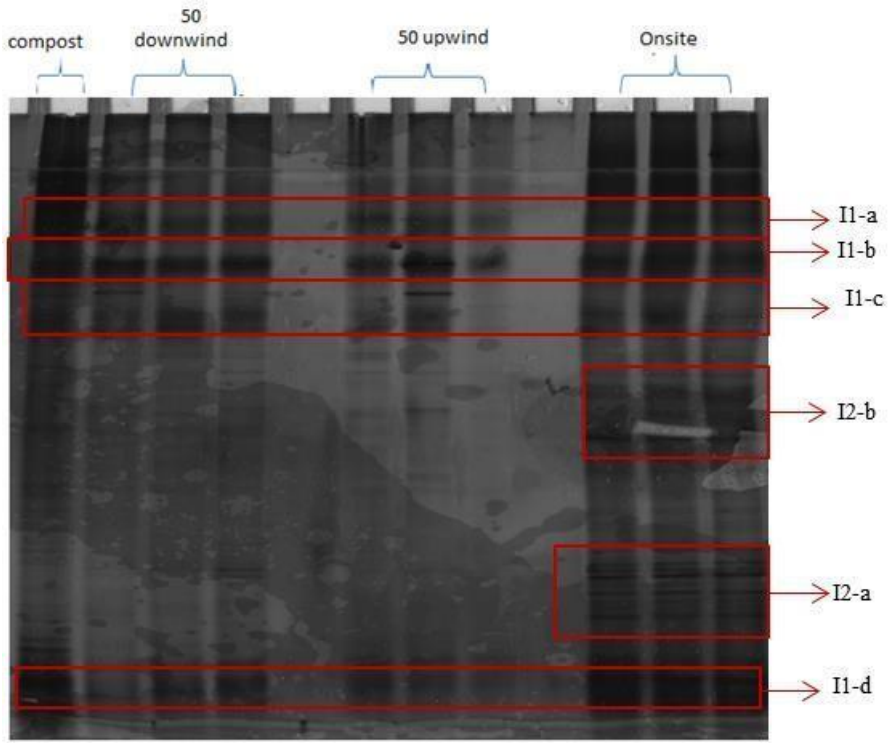

Figure 4.7 (a) DGGE profiles of bacterial 16S rDNA PCR amplicons of compost and bioaerosols from the Lahore compost facility, Lahore Pakistan.

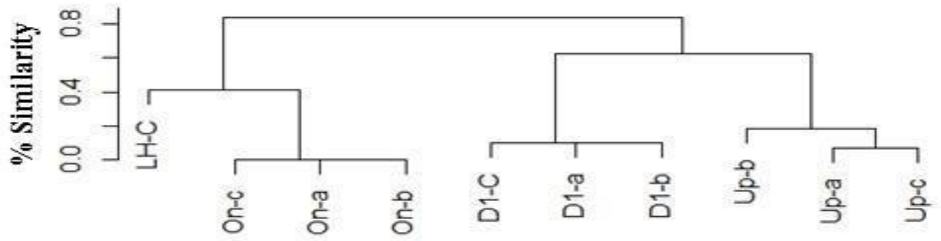

Figure 4.7 (b) Cluster dendrogram of the banding pattern in figure 4.7 (a) using a complete linkage (Jaccard cofficient). LH-C = compost; Onsite = On - a, b, c; 50 meters downwind = D1- a, b, c; 50 meters upwind = UP- a, b, c.

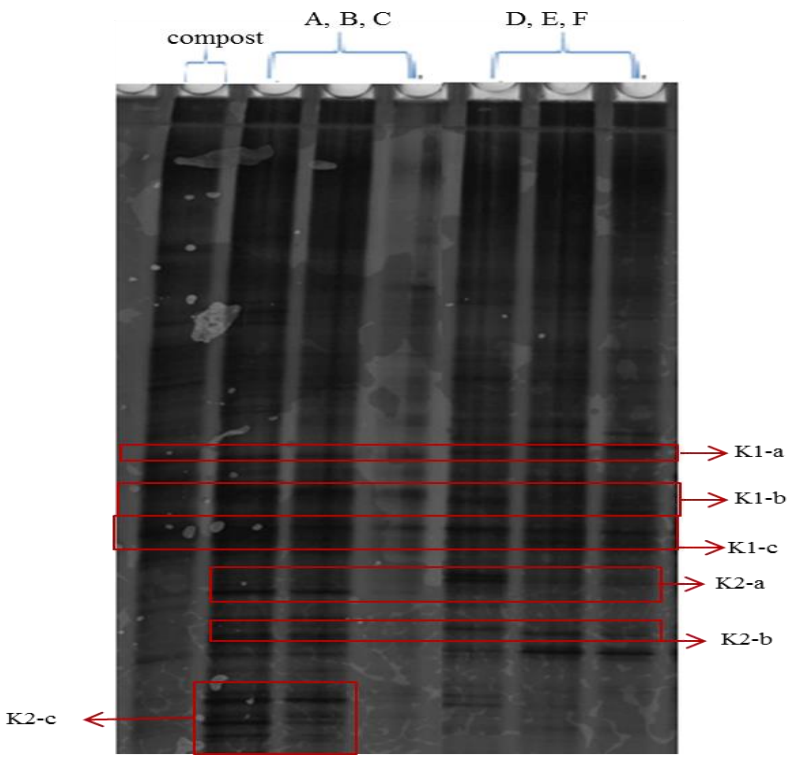

Figure 4.8 (a) DGGE profiles of bacterial 16S rDNA PCR amplicons of compost and bioaerosols from the village manure heap.

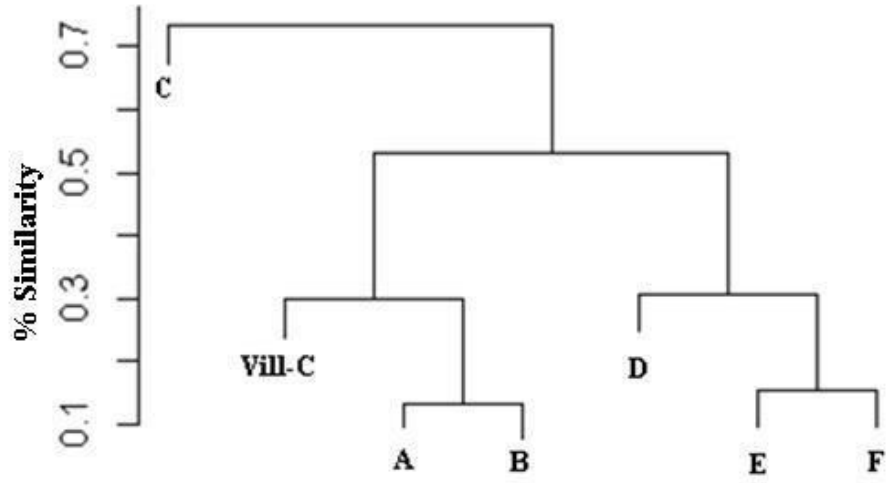

Figure 4.8 (b) Cluster dendrogram of the banding pattern in figure 4.8 (a) using a complete linkage (Jaccard coefficient) Vill-C = compost; A – F = bioaerosols samples.

4.2.5 Archaeal communities' analysis in the composting facilities and manure heap. Archaeal communities were not detected in all the samples of Waste Buster and Lahore compost facilities, Lahore Pakistan. However, the banding profile of archaeal 16S rDNA PCR amplicons of compost and bioaerosols from the manure heap showed the presence of similar fingerprints as specified by "Ar-a – Ar-c" (Fig 4.9a). Most of the compost banding profiles did not appear in the bioaerosol samples. Cluster analysis represents almost 40% similarity between the community compositions of the compost sample with that of bioaerosols (Fig 4.9b).

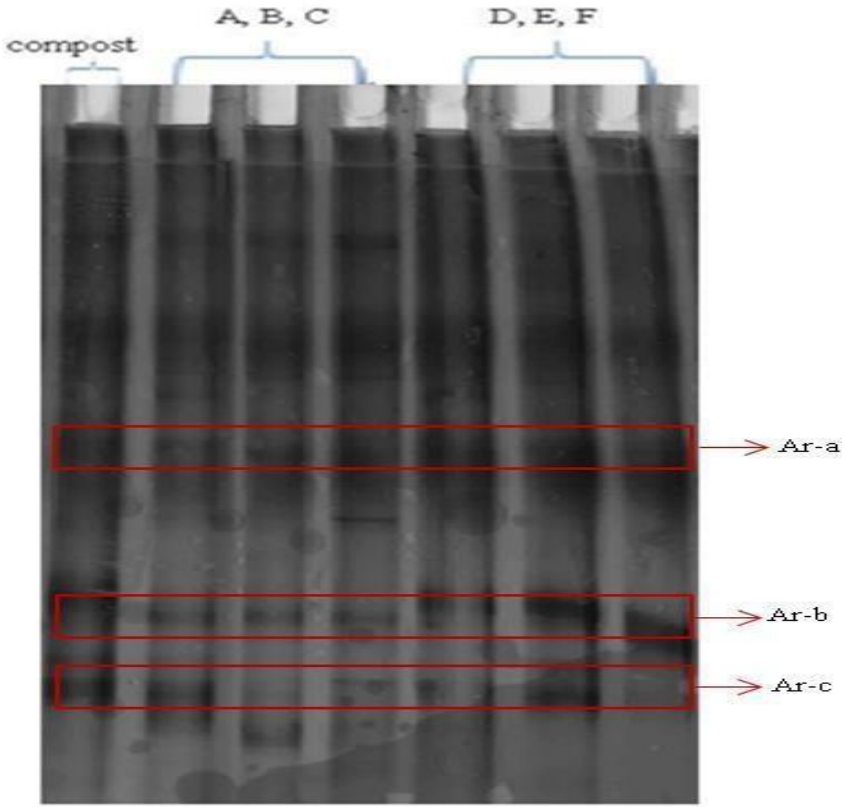

Figure 4.9 (a) DGGE profiles of archaeal 16S rDNA PCR amplicons of compost and bioaerosols from the village manure heap

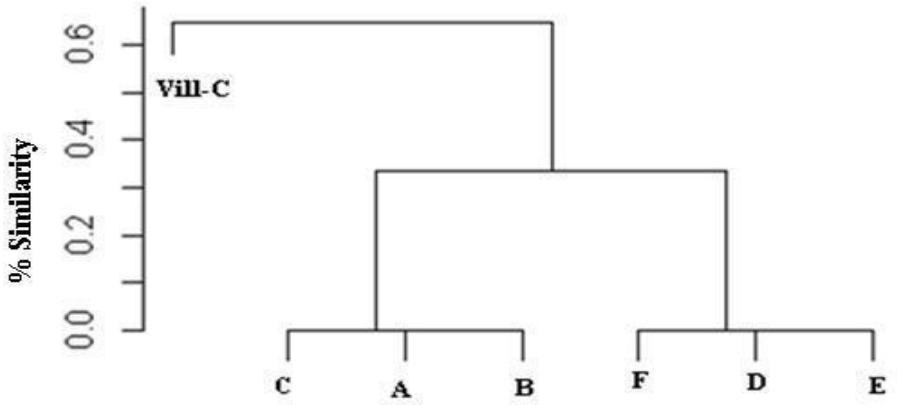

Figure 4.9 (b) Cluster dendrogram of the banding pattern in figure 4.9 (a) using a complete linkage (Jaccard coefficient). Vill-C = compost; A – F = bioaerosols samples.

4.2.6 Fungal community analysis in the composting facilities and manure heap.

PCR-DGGE analysis of fungal communities obtained from the Waste Buster facility indicated a diverse distribution of fungal communities among compost and bioaerosol samples. However, some of the fingerprints (H2-a – H2-b) appeared in all of the samples; whereas, "H1-a-H1-c indicated band profiles that are only found in aerosol samples (Fig 4.10a). Fungal flora of the compost sample is considerably different from the bioaerosols' microbial population, except for onsite fingerprints which showed a slight similarity with compost communities. Resemblance between the banding patterns indicated richness of some band profiles at 50 meters downwind that possibly reduced with increasing distance downwind. However, the fungal population of upwind samples showed some fingerprints in high abundance like onsite and downwind samples (H1-a) (Fig 4.10a). Cluster analysis of the banding profile of fungal communities revealed almost 50% similarity between compost and onsite samples. Almost 60% similarity was observed between 50 meters and 250 meters downwind samples. Conversely, the banding profiles of upwind samples indicated almost 30 % similarity with those downwind. Compost and onsite cluster showed 20% similarity with the cluster for both upwind and downwind samples (Fig 4.10b). PCR-DGGE analysis of fungal communities of Lahore compost indicated a diverse community distribution among samples. Some of the fingerprints were similar in all the samples as indicated by Figure 4.11a "J1-a & J1-b". However, onsite samples have more fungal diversity as compared with upwind and 50 meters downwind i.e. "J2-a & J2-b". Some of the onsite band profiles were also similar with the compost species (J4-a), whereas, "J3-a" represented compost specific fingerprints (Fig 4.11a). A cluster dendrogram of fungal PCR amplicon illustrated a 50% similarity between compost microbial communities and those onsite. However, the fungal population of downwind and upwind samples shared almost 60% similarities with each other, except for the D1-b which represented lesser fungal species (Fig 4.11b). The DGGE gel showed the presence of almost similar fingerprints as shown in Figure 4.14a "L1-a – L1-c" of the manure heap. Some of the fingerprints appeared only in

the aerosol samples as specified by “L2-a – L2-b”. The banding pattern also indicates a dissimilar fingerprint distribution in the compost as compared with the aerosols (Fig 4.12a). Cluster analysis represents almost 20% similarity of fungal communities between compost and aerosol samples (Fig 4.12b).

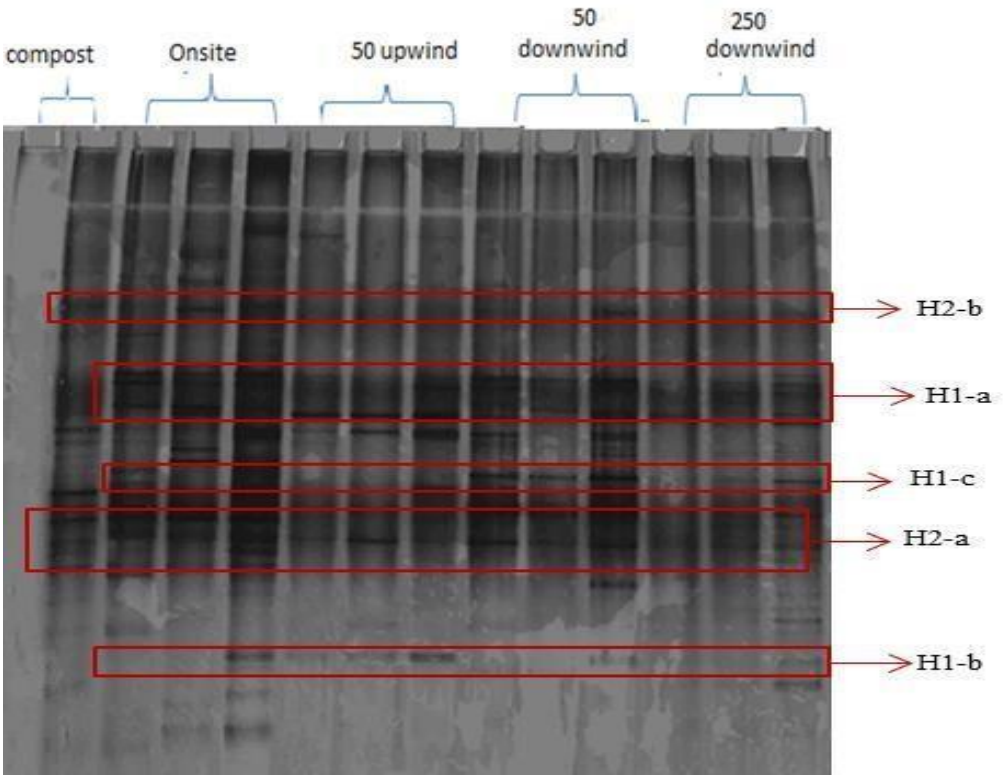

Figure 4.10 (a) DGGE profiles of fungal ribosomal DNA (ITS2 region) PCR amplicons of compost and bioaerosols from the Waste Buster composting facility, Lahore Pakistan.

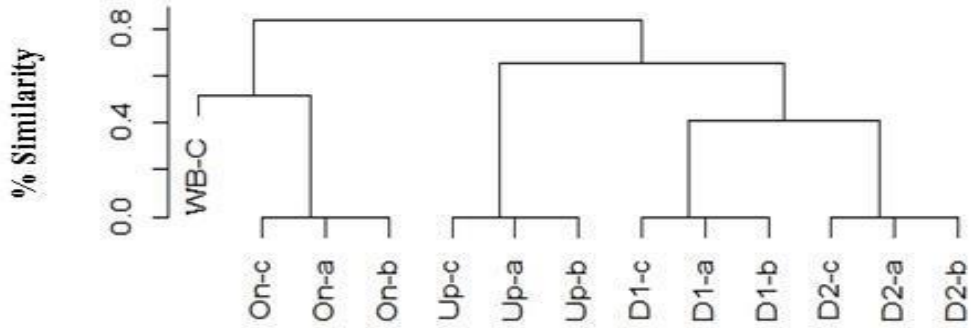

Figure 4.10(b) Cluster dendrogram of the banding pattern in figure 4.11 (a) using a complete linkage (Jaccard cofficient). WB-C = compost; Onsite = On - a, b, c; 50 meters downwind = D1- a, b, c; 250 meters downwind = D2- a, b, c; 50 meters upwind = UP- a, b, c.

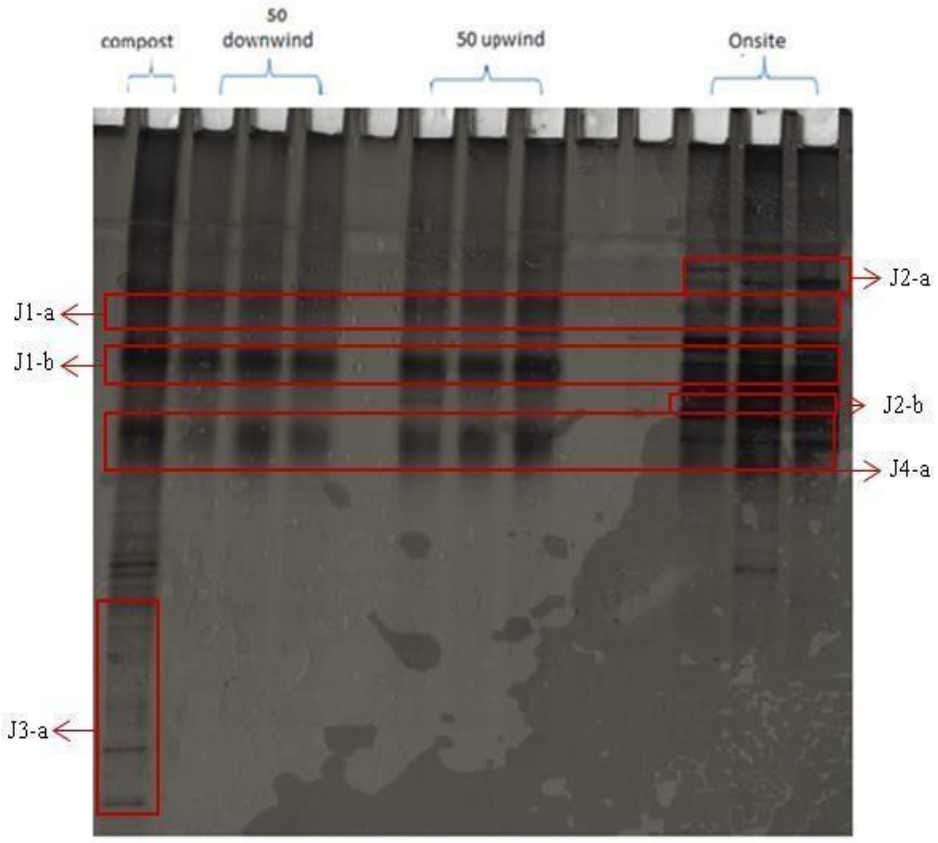

Figure 4.11 (a) DGGE profiles of fungal ribosomal DNA (ITS2 region) PCR amplicons of compost and bioaerosols from the Lahore compost facility, Lahore Pakistan.

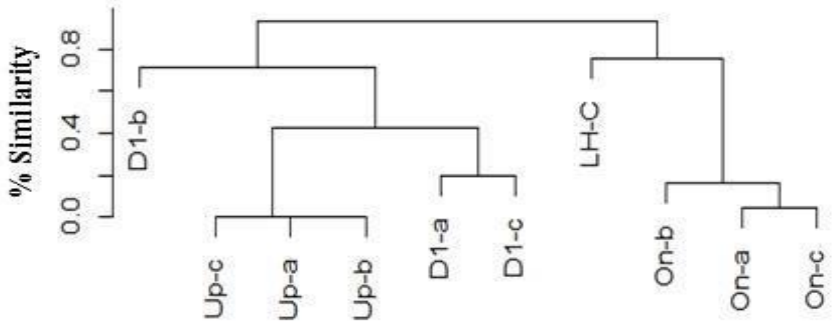

Figure 4.11 (b) Cluster dendrogram of the banding pattern in figure 4.12 (a) using a complete linkage (Jaccard cofficient). LH-C = compost; Onsite = On - a, b, c; 50 meters downwind = D1- a, b, c; 50 meters upwind = UP- a, b, c.

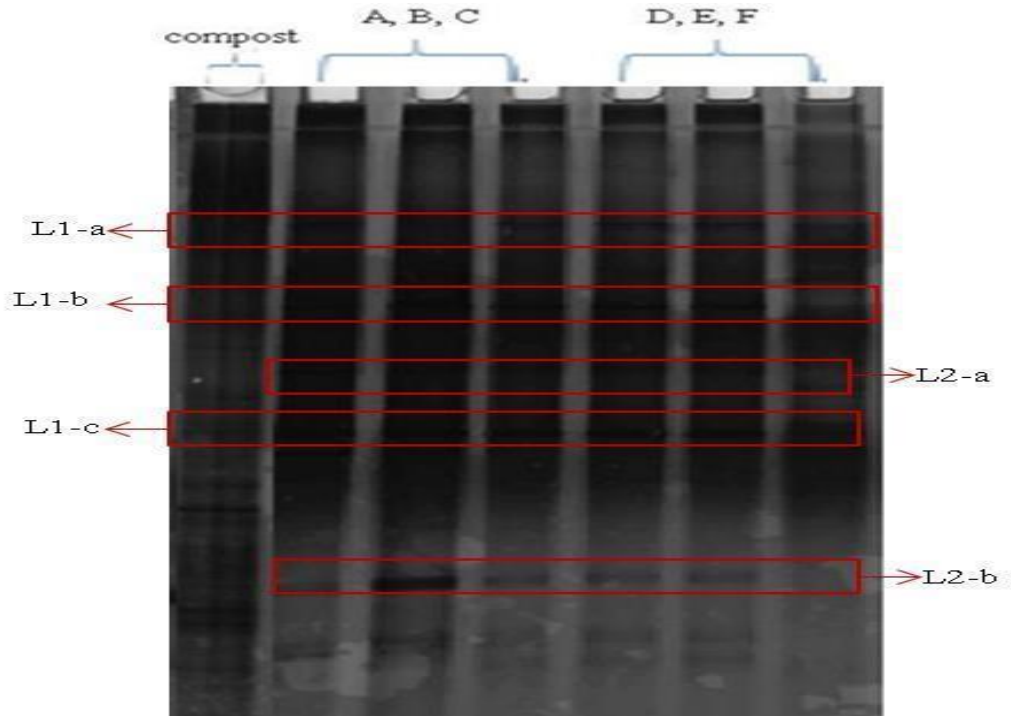

Figure 4.12 (a) DGGE profiles of fungal ribosomal DNA (ITS2 region) PCR amplicons of compost and bioaerosols from the village manure heap.

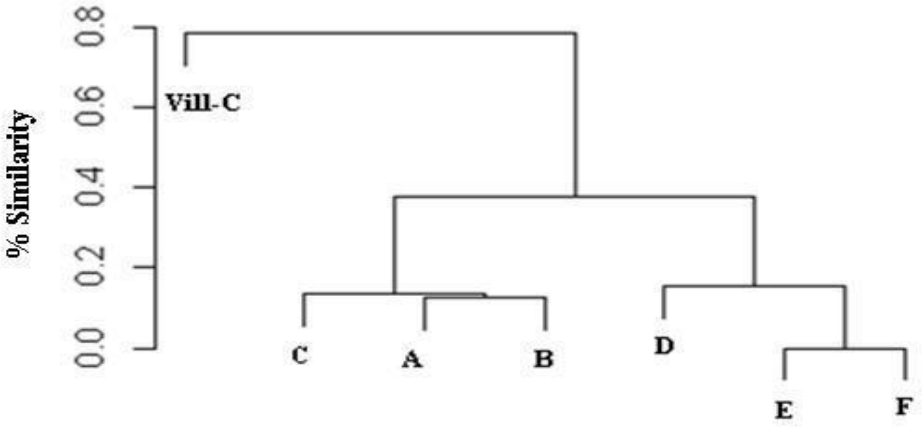

Figure 4.12 (b) Cluster dendrogram of the banding pattern in figure 4.13 (a) using a complete linkage (Jaccard coefficient). Vill-C = compost; A – F = bioaerosols samples.

4.3 Discussion

The main aim of this study was to evaluate the dispersal and microbial community dynamics of compost associated bioaerosols in the proximity of sites and that this could eventually help in the evaluation of hazards posed by bioaerosols. The dispersal of bioaerosols from composting sites depends on various factors i.e. physical and chemical properties of bioaerosols, site topography and climatic settings (Wery, 2014). Although higher microbial concentrations were recorded onsite a decrease in the abundances of bioaerosols was observed with increasing distance across each composting facility. This configuration of bioaerosols dispersion has been discussed earlier in chapter 3.

Q-PCR data indicated onsite abundance of *S. rectivirgula* between $10^6 – 10^7$ copies of 16S rDNA m^{-3} at both of the composting facilities. These results are similar with previously (Schafer *et al.,* 2013), reported values of 10^2 to 10^7 cells of *S. rectivirgula* in a composting plant. Similarly, whilst quantifying *Saccharopolyspora sp.* onsite of the composting site Le Goff *et al.* (2011) reported 2 x 10^6 to 4 x 10^7 copies of 16S rDNA m^{-3}. The abundances tend to decrease along increasing distance from both sites. However, the abundance obtained at 250 m downwind of the Waste Buster composting site was higher than that of 50 m upwind. The results obtained from the Birch Airfield composting site during different seasons indicated the abundances of *S. rectivirgula* i.e. 9.6 x 10^8 16S rRNA gene copies/m^3 (summer); 2.3 x 10^8 16S rRNA gene copies/m^3 (autumn) and 7.8 x 10^7 16S rRNA gene copies/m^3 (winter) respectively. Comparing these results with abundance results obtained from Pakistan composting sites illustrates higher abundance in summer and autumn for a UK composting site as compared with the Pakistan composting facility. However, abundance obtained in the winter season was similar with that of the Pakistan sites.

It has been reported that the concentrations of bioaerosols at one particular site differ on an hourly basis and can significantly change by more than 10 fold (Neef *et al.,* 1999). The abundance of bacteria was higher onsite in the Lahore compost facility as compared with the

Waste Buster and village manure heap; however, its abundance was less at 50 m upwind anddownwind compared to the Waste Buster facility. At the time of onsite sampling at the Lahore compost site, only shredding was taking place; whereas at the Waste Buster composting facility screening and turning of the compost was being carried out. However, this pattern can be explained by topography of the Lahore compost site which was also surrounded by an open dumping facility whereby continuous dumping of waste and manoeuvring of vehicles would result in increased dispersion of bioaerosols and this is assumed to influence the bacterial abundance. While bioaerosol abundance declined downwind of the composting facilities; however, less consistency was observed in the configuration of a reduction in abundance from 50 m to 250 m downwind of the composting facility which has also been previously reported by Stagg *et al.* (2010). This suggests the influences of turbulence, wind and particle size in this discrepancy. Most of emitted bioaerosols from the composting sites are small (0.5-1 µm) with a smaller fraction of larger particles (Vestlund *et al.,* 2014). Colls (2002) suggested that smaller airborne particles travel further due to the increased thermal energy resulting in rapid dispersal. The dispersion of bioaerosols is also dependent on the size distribution of the particles, as relatively small particles can travel large distances comparted to larger particles (Byeon *et al.,* 2008). Further, single cell bacteria emitted from compost are suggested to be dominant at 50 meters downwind of the site compared with large sized bacterial aggregates and due to their greater air residence time these can travel large distances relative to bacterial aggregates (Galès *et al.,* 2015). This difference in abundance between composting sites of the two different regions (UK & Pakistan) can be for many reasons, for example scale of composting activity, composition of the waste material, bioaerosol dispersion activities and ambient atmospheric settings. It has been suggested that the amount of microorganisms produced during composting can fluctuate from one site to another depending on the scale and type of composting procedures (Swan *et al.,* 2003). The UK composting facility is capable of recycling 30,000 tonnes green waste annually; whereas, the Waste Buster site is capable of recycling approximately 20,000 tonnes organic waste per annum.

Regarding the geographic features of the composting facilities, the topography of the site is also proposed to influence the dispersal of the bioaerosols (Wery, 2014). The terrain of the UK composting site is entirely different from the Pakistan composting sites. The UK site is surrounded by fields and a vast agricultural area; whereas, the Pakistan sites are surrounded by residential and industrial areas. This would also effect the dispersion of the bioaerosols downwind of the site. Bell and Treshaw (2002) reported higher emission of bioaerosols from complex topography due to the influence of constant atmospheric settings. Similarly, the uneven surfaces of the composting site cause higher transmission of bioaerosols due to higher frictional drag (Bell & Treshaw, 2002). Moreover, the occurrence of low microbial abundance at Pakistan's sites as compared with the UK facility can be explained from wind speed as it is suggested to increase the dispersal of bioaerosols. Data showed that the wind speed was negatively correlated with the microbial abundance in Pakistan; whereas, it was positively correlated in the UK sampling results except in summer. The lower wind speed (0.2 m/s) can possibly be the reason of lesser microbial abundance even though the atmospheric conditions were drier as compared with the UK as it has been reported that an increase in wind speed leads to the increased dispersion of bioaerosols across composting sites (Oke, 1987; Jones and Harrison, 2004; Swan *et al*, 2003).

Concerning bacterial diversity at the Waste Buster site, DGGE analysis indicated the existence of more microbial communities at 250 m downwind than upwind. The presence of significant microbial flora at 250 m downwind may be possibly due to onsite activities i.e. turning, shredding and screening. However, less consistency in dispersion was observed from 50 meters to 250 meters downwind at the Waste Buster site. This may be influenced by particle size, turbulence and prevailing winds (Stagg *et al.,* 2010). Concerning onsite activities, it has been reported (Schlosser *et al.,* 2009; Pankhurst, 2010) that shredding would not generate bioaerosols due to fewer microorganisms being associated with fresh waste, as compared with turning and screening that would produce higher emissions of bioaerosols. Further, a higher fungal community distribution was observed as compared with bacterial communities at 250 meters downwind of the Waste Buster site. This reflects

the high dispersal and survival intensity of fungal aerosols than bacteria. Generally, the Waste Buster site represented a higher fungal community distribution than the Lahore compost site. This might again be due to the composting activities onsite at Waste Buster's as compared with Lahore compost where shredding of compost was conducted. Further, during the period of no onsite activity, downwind concentrations were reported to be similar with the upwind levels (Pankhurst, 2010). Previous published literature has indicated an increase in the concentration of bioaerosols due to onsite composting activities (Clark *et al.,* 1983; Taha *et al.,* 2006). The low amount of microbial concentration at the Lahore compost site comparative to Waste Buster's can be attributed to site activities.

The archaeal community was only found in bioaerosols of the manure heap as compared with the field scale composting facilities in both the UK and Pakistan. Previously, studies have assessed the anaerobic archaeal population in the composting environments (Chouari *et al.,* 2005; Narihiro *et al.,* 2007; Shin *et al.,* 2010; Tabatabaei *et al.,* 2010; Yamamoto *et al.,* 2011). Yet, there is a limited amount of published data to characterize the microbial diversity of airborne archaea. In this research, no archaeal population was detected in the samples from the field scale composting facilities and this suggests the aerobic environment of the composting activity. However, anoxic settings during waste decomposition result in the occurrence of anaerobic methane producing archaeal communities. Thummes *et al.* (2007) reported on the emission of thermophilic methanogenic archaea during turning of compost and found the presence of *Methanosarcina thermophila*, *Methanoculleus thermophilus,* and *Methanobacterium formicicum* in the bioaerosols. It has been suggested that the common archaea found in the anaerobic environment belongs to the orders *Methanobacteriales*, *Methanomicrobiales* and *Methanosarcinales* which are associated with bacteria in the digestion process (Stams *et al.,* 2009). In another study Nehmé *et al.* (2007) indicated the presence of high airborne archaeal communities in bioaerosols emitting from swine confinement buildings and documented the abundance of *Methanosphaera stadtmanae* (94.7%) in the bioaerosols.

4.4 Conclusion:

It was anticipated that due to warm meteorological settings of Pakistan, there would be higher microbial abundance, however results were contradictory with the hypothesis. Maximum abundance was found onsite as compared with upwind and downwind of the composting facilities. Statistical significant difference (p-value < 0.05) of mean abundances was observed across composting sites. No significant relationship was found between meteorological parameters and microbial abundance.

Chapter 5 A laboratory scale experiment to assess the effect of temperature and moisture content on bioaerosols emitted from compost

5.1 Introduction

Temperature and moisture have been regarded as significant factors in the composting process that affects the microbial flora of the compost throughout the process. The effect of temperature on the microbial composition and abundance of compost has been previously reported in various laboratory based studies (Macgregor *et al.*, 1981; Strom, 1985; Nakasaki *et al.*,1985; Tiquia *et al.,* 1996). It is regarded as a significant factor in the efficiency of the microbial activity during composting as microbial metabolism and composition and abundance are highly temperature dependent (Liang *et al.,* 2003). Researchers (Suler & Finstein, 1977; Nakasaki *et al.*, 1985; Strom, 1985) have indicated different ranges of optimum temperature for the effective degradation of organic material. However, there is a lack of literature on the influence of temperature on the emission of compost associated aerosols.

Moisture content is a significant factor in the composting process as it aids in the transportation of nutrients to the microbial flora of the compost. It has been suggested that low moisture levels of the compost can lead to desiccation (Debertoldi *et al.*, 1983) leading to the inhibition of biological activity; whereas higher moisture content can impede oxygen availability for the microbial population thus resulting in anaerobic conditions (Miller *et al*., 1989). Previously, different ranges of optimal moisture contents have been identified for the stable composting process Suler & Finstein (1977) and McKinley & Vestal (1984) specified an optimum moisture level of 50-60% , whereas, Richard *et al.* (2002) indicated a 50-70% was moisture content suitable for composting.

This chapter aims to investigate the effect of temperature and moisture content on the microbial community composition and abundance of bioaerosols emitted from compost. It is hypothesized that a difference in microbial community composition and abundance will occur during different stages of temperatures and moisture contents whereby higher

microbial abundance will occur in bioaerosols released from compost incubated at 33°C with 60% moisture content. The findings are intended to help understand the impact of ambient temperature and moisture level (humidity) on the microbial community composition and abundance. Further, it would be helpful in understanding the interactions of microbial diversity of the bioaerosols with the meteorological variables.

5.2 Results

The experimental set up of the laboratory scale temperature and humidity investigation is presented in Table 5.1.

Table 5.1 Description of bioaerosol sampling operation from a compost box at different temperatures and moisture contents

Incubation temperature °C	Starting moisture content %	Sampling days	Sample ID	Humidity%
33	40	7	1st a, b, c	16
33	40	14	2nd a, b, c	35
33	40	21	3rd a, b, c	67
33	40	28	4th a, b, c	88
33	60	7	1st a, b, c	22
33	60	14	2nd a, b, c	48
33	60	21	3rd a, b, c	75
33	60	28	4th a, b, c	94
33	80	7	1st a, b, c	28
33	80	14	2nd a, b, c	54
33	80	21	3rd a, b, c	83
33	80	28	4th a, b, c	97
33	60	7	1st a, b, c	17
33	60	14	2nd a, b, c	45
33	60	21	3rd a, b, c	71
33	60	28	4th a, b, c	93
22	60	7	1st a, b, c	12
22	60	14	2nd a, b, c	34
22	60	21	3rd a, b, c	64
22	60	28	4th a, b, c	82
12	60	7	1st a, b, c	7
12	60	14	2nd a, b, c	29
12	60	21	3rd a, b, c	57
12	60	28	4th a, b, c	74

5.2.1 Effect of temperature on bacterial abundance in bioaerosols from compost

When compost was incubated at 33°C the abundance of total bacteria at day 7 was increased by day 14. However, bacterial abundances decreased by day 21 to day 28 (Fig 5.1, Table 5.2). An increase of one order of magnitude was observed between day 7 and day 14; whereas a decrease of three orders of magnitude was observed between day 21 to day 28. During the experiment, the humidity inside the compost box increased from 17% in the first week to 93% by week four (Table 5.1). The abundance of total bacteria at 22°C indicated an increase of two orders of magnitude between day 7 and day 14. Nevertheless, a decline of one order of magnitude was observed between day 14 to day 21 and two orders of magnitude between day 21 to day 28 (Fig 5.1, Table 5.2).During the experiment, the humidity inside the compost box increased from 12% in the first week to 84% by week four (Table 5.1). The abundance of bacteria obtained at 12°C indicated a decrease of one order of magnitude between 14 and day 21 and between day 21 and day 28. This decrease continued by day 21 and day 28 (Fig 5.1, Table 5.2). Humidity increased continuously throughout the experiment from 7% in day 7 to 74% by day 28 (Table 5.1).

Table 5.2 Quantification of total bacteria in bioaerosols emitted from compost at different temperatures.

Sampling days	33°C	22°C	12°C
7	1.15×10^{9}	5.8×10^{8}	8.9×10^{9}
14	6.1×10^{10}	2.9×10^{10}	2.3×10^{9}
21	3.4×10^{10}	1.2×10^{9}	4×10^{8}
28	2.1×10^{7}	8.5×10^{7}	8.4×10^{7}

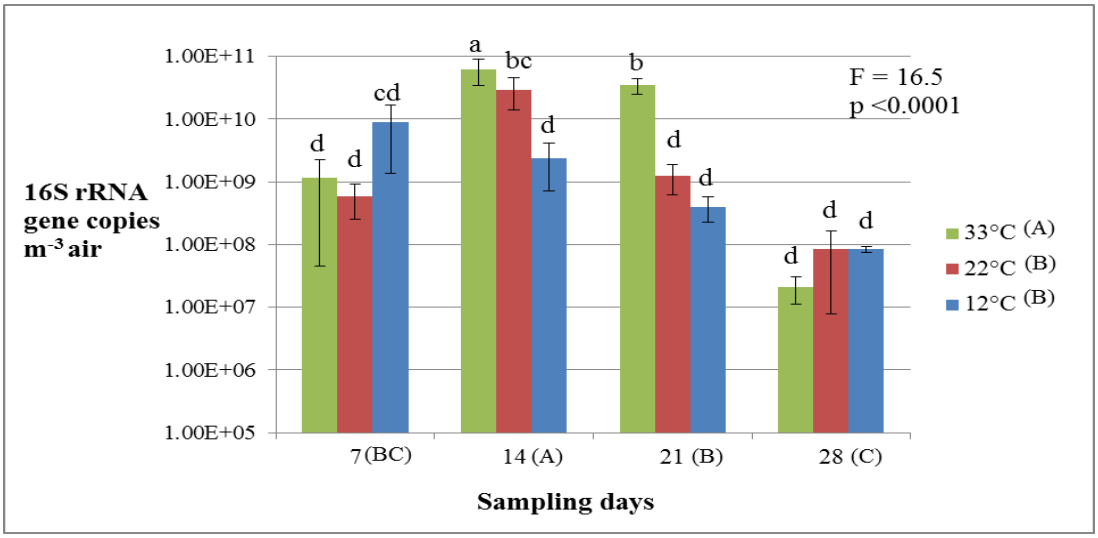

Figure 5.1 Abundance of bacteria in bioaerosols emitted from compost at different temperatures. 7 = day 7; 14 = day 14; 21 = day 21 and 28 = day 28 of the composting experiment. Error bars represent standard error of mean (*n*=3). Significant differences are shown by ANOVA and Tukey's HSD. Different letters in brackets after temperature, sampling days or above bars indicate significant differences at 0.05 confidence interval.

5.2.2 Effect of temperature on abundance of *Saccharopolyspora rectivirgula* in bioaerosols from compost

The abundance of *Saccharopolyspora rectivirgula*, at day 7 found in compost incubated at 33°C was increased to one order of magnitude by day 14. However, a decline in abundance of one and two orders of magnitude was observed from day 21 to day 28 of the experiment respectively (Fig 5.2, Table 5.3). When incubated at 22°C the abundance of *Saccharopolyspora rectivirgula* was increased by 3 order of magnitude by day 14. However, a decrease in the abundances was observed from day 21 to day 28 i.e. decrease from 1 order of magnitude to 2 order of magnitude (Fig 5.2, Table 5.3). At 12°C, the abundance of *Saccharopolyspora rectivirgula* was 1 order of magnitude day 14. However, a swift decline in the abundances was observed on day 21 i.e. decrease of 2 order of magnitude followed a slow decrease of 1 order of magnitude by day 28 of the experiment i.e. 7.8 x 10^4 16S rRNA gene copies/m^3 (Fig 5.2).

Table 5.3 Quantification of *Saccharopolyspora rectivirgula* in bioaerosols emitted from compost at different temperatures.

Sampling days	33°C	22°C	12°C
7	7.6×10^6	5.6×10^5	8.9×10^6
14	3.4×10^7	8.7×10^8	6.8×10^7
21	4.3×10^5	8.2×10^7	6.3×10^5
28	5.24×10^4	8.6×10^5	7.8×10^4

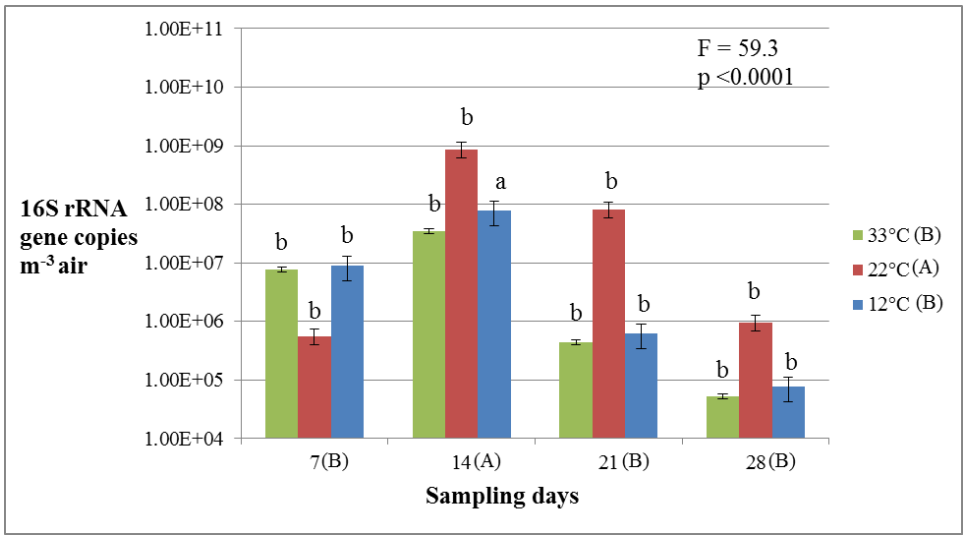

Figure 5.2 Abundance of *Saccharopolyspora rectivirgula* in bioaerosols emitted from compost at different temperatures. 7 = day 7; 14 = day 14; 21 = day 21 and 28 = day 28 of the composting experiment. Error bars represent standard error of mean (*n*=3). Significant differences are shown by ANOVA and Tukey's HSD. Different letters in brackets after temperature, sampling days or above bars indicate significant differences at 0.05 confidence interval.

5.2.3 Effect of temperature on bacterial community dynamics in bioaerosols from compost

PCR-DGGE analysis of bacterial 16S rRNA genes from bioaerosols emitted from compost at 33°C indicated fewer bands on day 7 (Figure 5.3a "M1-a- M1-c" specifies fingerprints that were abundant on day seven were less abundant in the corresponding days); whereas, higher microbial diversity was recorded in samples collected on day 14 as "M2-a – M2-c" represent that fingerprints those were less abundant on the day seven were rich on day 14. Further, M3-a- M3-b indicates band profiles that occurred only in the second week (day 14). Some of the band profiles of the third week (day 21) were similar with the first (day 7) and second week (day 14) bands structure as indicated by "M4-a – M4-b". Also, "M5-a" represents one of the fingerprint with high abundance in the third week (Fig 5.3a).

Band profiles showed a decrease in the number of fingerprints in the samples taken on the third (day 21) and fourth weeks (day 28) as faint bands (M6-a – M6-c) represent fingerprints that were rich in previous weeks became less abundant. "M7-a" indicates band profiles that were abundant in first two weeks (day 7 to 14), decreased on day 21 and showed richness again in the fourth week (day 28) (Fig 5.3a). Hierarchal cluster analysis showed that the fingerprint structure of the day 14 and 21 samples was almost 80% similar with each other. The banding profile indicated that cluster of day 28 shared almost 50% similarity with the cluster of days 14 and 21. However, the band profile for day seven was diverse and indicated 20% similarity with the fingerprint structure of days 14, 21 and 28 (Fig 5.3b).

Bacterial 16S rDNA PCR amplicons of bioaerosols from compost at 22°C indicated low fingerprints (Figure 5.4a O1-a- O1-b) on day seven; whereas, greater bands profiles were recorded in samples collected on day 14 as indicated by "O2-a – O2-d". However, the banding profiles showed a decrease in the number of fingerprints in the samples taken on week three (day 21) and four (day 28) (Fig 5.4a). Some fingerprints were represented across all of the samples; however their abundance was dissimilar due to the faintness of the bands (O3-a). In one of the samples of day 28 (4^{th} – a) the bright bands specifies the presence of

diverse fingerprints as compared with other samples i.e. O4-a. Hierarchal cluster analysis revealed that the fingerprint structure on day 14 was diverse from the samples of the other weeks. The banding profile indicated about 20 – 30% similarity among the cluster of day 14 day and clusters of days 7, 21 and 28 (Fig 5.4b).

Bacterial community analysis from the DGGE profiles in bioaerosols from compost at 12°C represented the presence of diverse fingerprint distribution (fig 5.5a). Some of the band profiles that appeared on day 7 were not observed in days 14 and 21 and appeared again on day 28 as represented by (Q4a- Q4-b); however, some of the fingerprints were present in all of the samples while differences were recorded among their abundances in different days as "Q1-a" indicates fingerprints that were abundant in all the samples; whereas, in "Q2-a- Q2-e" faint bands specifies less abundance of fingerprint. Cluster analysis of the banding patterns indicated almost 70% similarity between days14 and 21 which together shared almost 50% similarity with the fingerprint structure of the day seven samples. The bacterial signature of days 7, 14 and 21 indicated 20% similarity with day 28 day (Fig 5.5b).

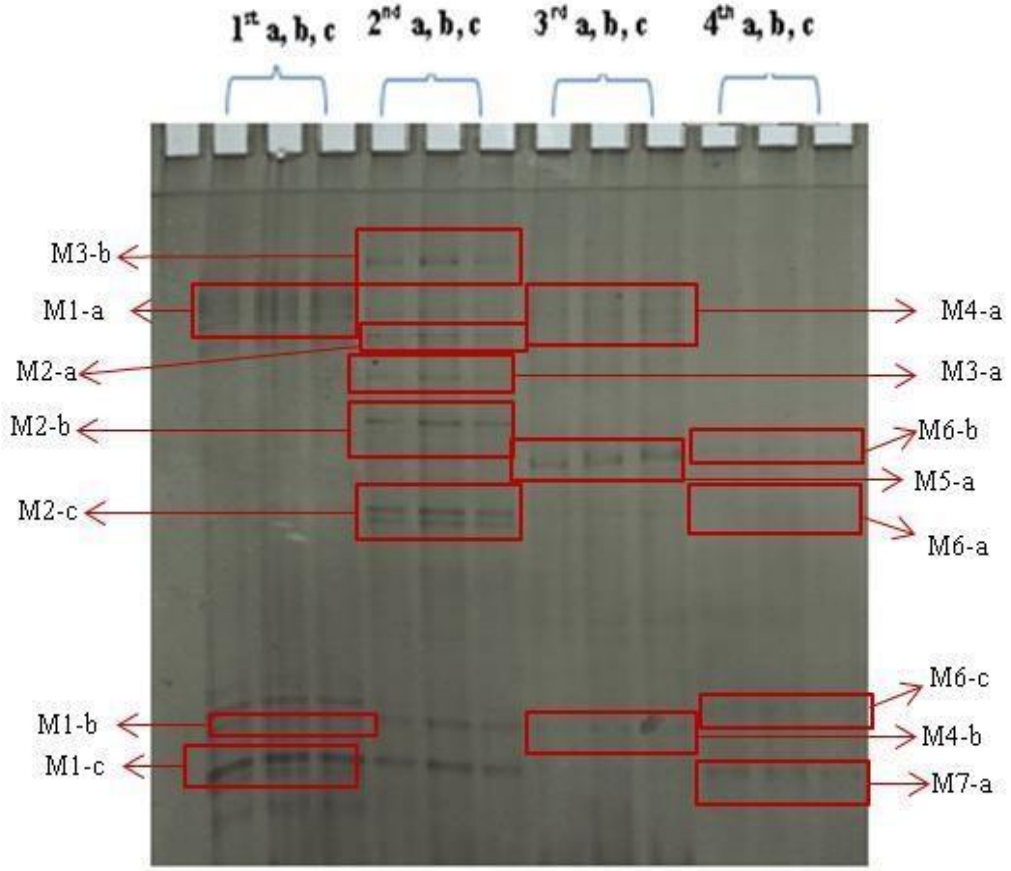

Figure 5.3 (a) DGGE profiles of bacterial 16S rDNA PCR amplicons of bioaerosols from the compost box at 33°C.

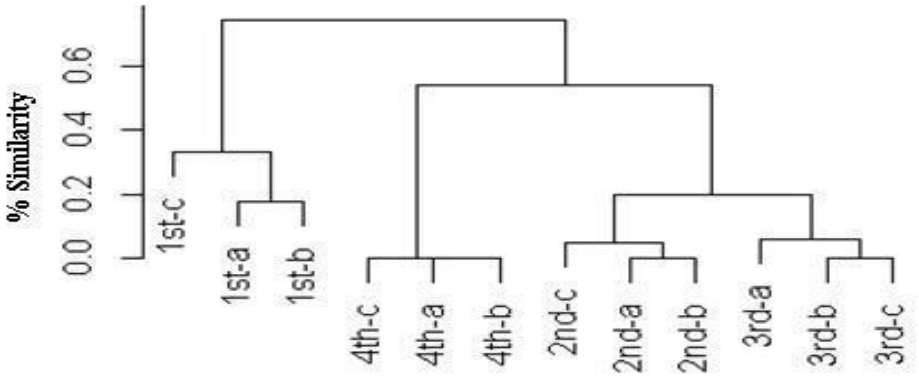

Figure 5.3 (b) Cluster dendrogram of the banding pattern in figure 5.3 (a) using a complete linkage (Jaccard cofficient). 1st a, b, c = day 7; 2nd a, b, c = day 14; 3rd a, b, c = day 21; 4th a, b, c = day 28.

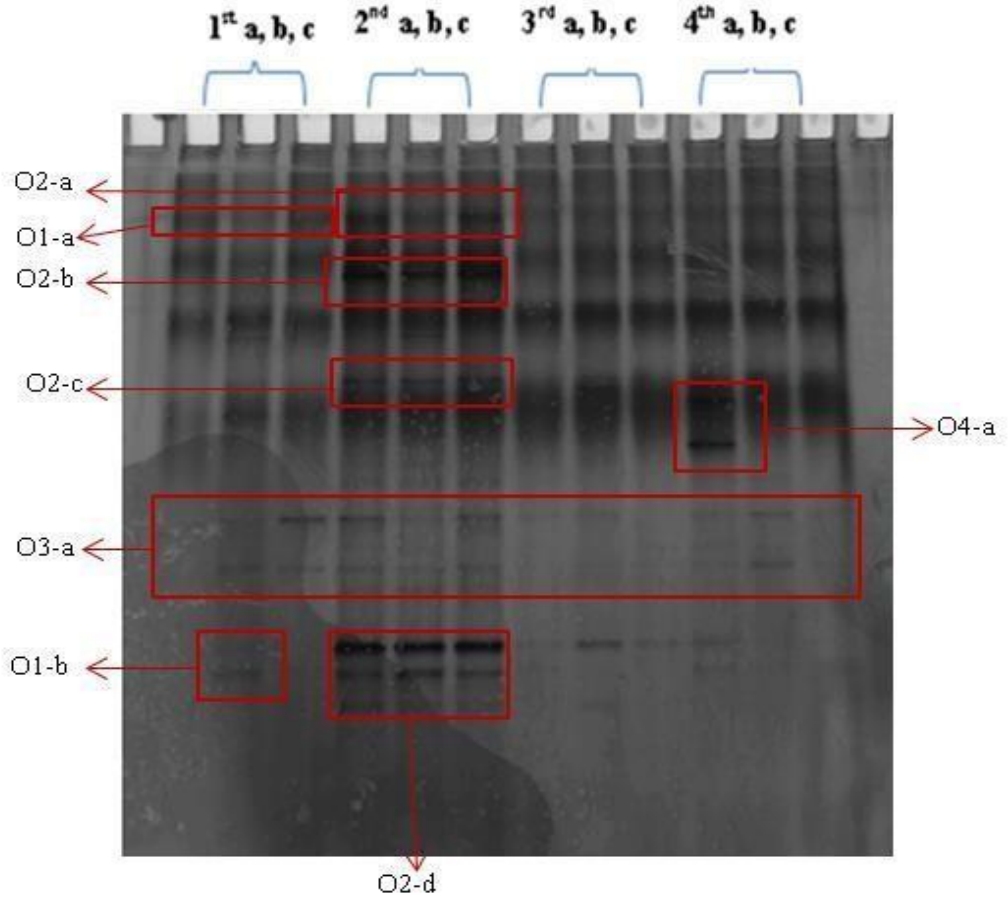

Figure 5.4 (a) DGGE profiles of bacterial 16S rDNA PCR amplicons of bioaerosols from the compost box at 22°C.

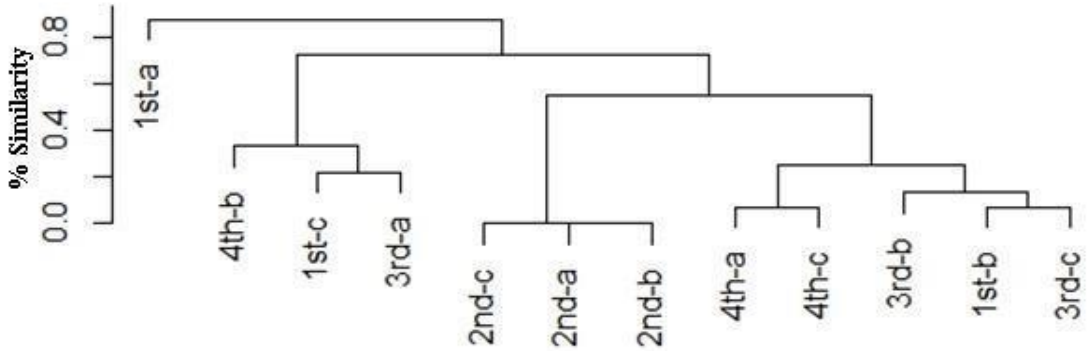

Figure 5.4 (b) Cluster dendrogram of the banding pattern in figure 5.4 (a) using a complete linkage (Jaccard cofficient). 1st a, b, c = day 7; 2nd a, b, c = day 14; 3rd a, b, c = day 21; 4th a, b, c = day 28.

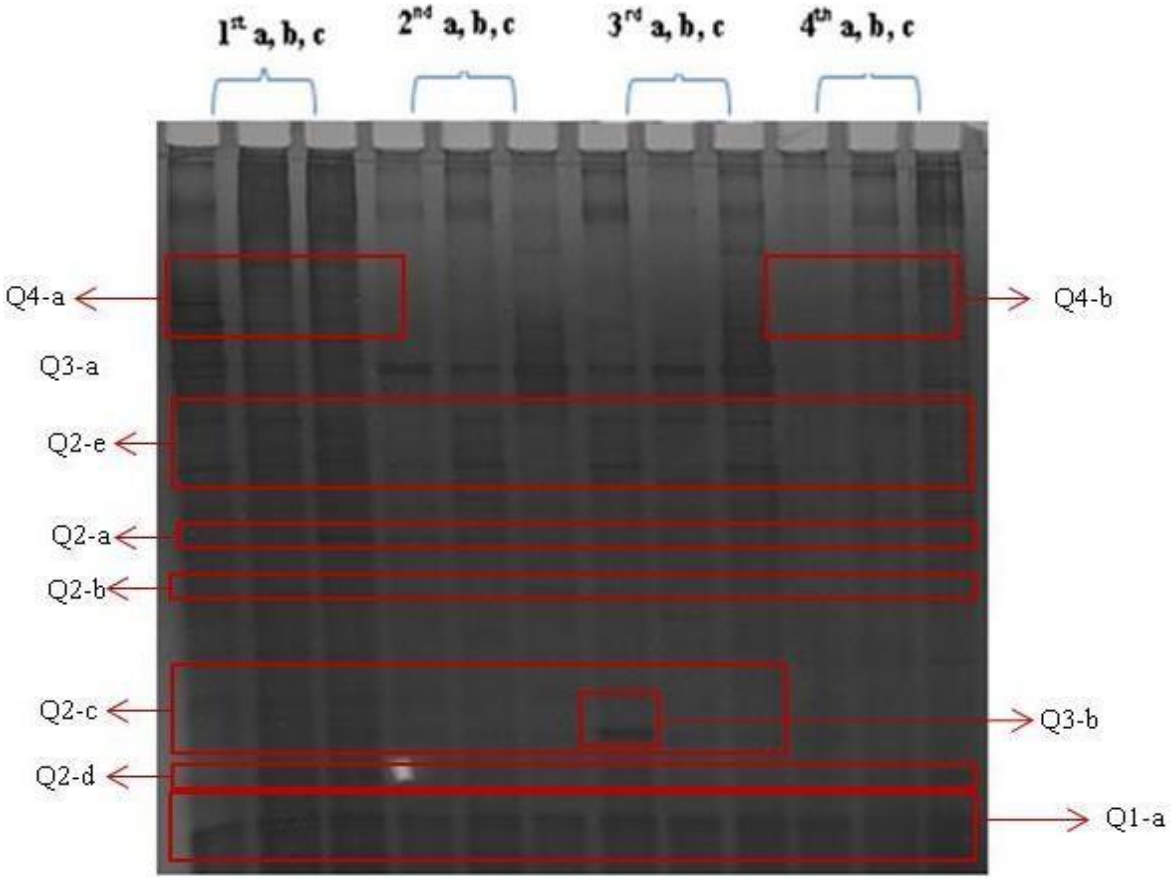

Figure 5.5 (a) DGGE profiles of bacterial 16S rDNA PCR amplicons of bioaerosols from the compost box at 12°C.

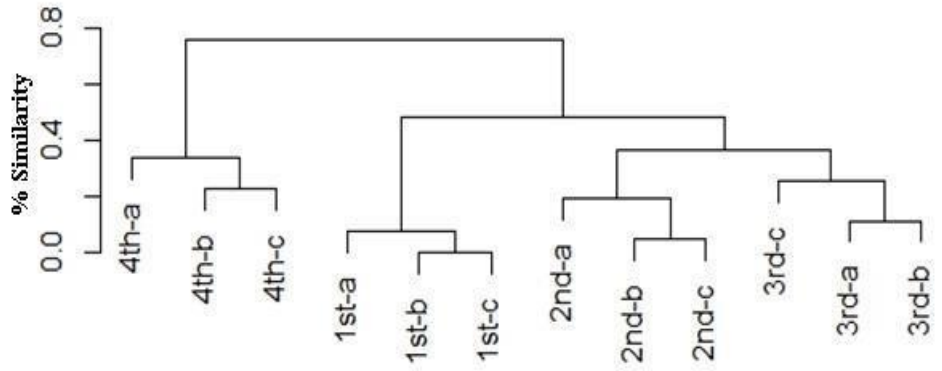

Figure 5.5 (b) Cluster dendrogram of the banding pattern in figure 5.5 (a) using a complete linkage (Jaccard cofficient). 1st a, b, c = day 7; 2nd a, b, c = day 14; 3rd a, b, c = day 21; 4th a, b, c = day 28.

5.2.4 Effect of temperature on abundance of *Aspergillus fumigatus* in bioaerosols from compost

The abundance of *Aspergillus fumigatus* found in compost incubated at 33°C indicated an increase of one order of magnitude from day 7 to day 14. The abundance started to decrease to one order of magnitude by day 21 and three orders of magnitude till day 28 respectively (Table 5.4, Fig 5.6). Humidity increased continuously throughout the experiment from 17% in the first week to 93% in the fourth week (Table 5.1). At 22°C, an increase of one order of magnitude was observed from the day 7 to day 14 that decreased by one order of magnitude by day 28 (Table 5.4, Fig 5.6). *Aspergillus fumigatus* abundance at 12°C increased from day 7 to day 14 by one order of magnitude. However, abundance decreased to one order of magnitude by day 28 (Table 5.4, Fig 5.6).

Table 5.4 Quantification of *Aspergillus fumigatus* in bioaerosols emitted from compost at different temperatures

Sampling days	33°C	22°C	12°C
7	8.3×10^8	3.7×10^6	2.4×10^5
14	1.8×10^9	4.9×10^7	3.7×10^6
21	2.6×10^8	2.3×10^6	9.6×10^5
28	7.5×10^5	3.07×10^6	2.8×10^5

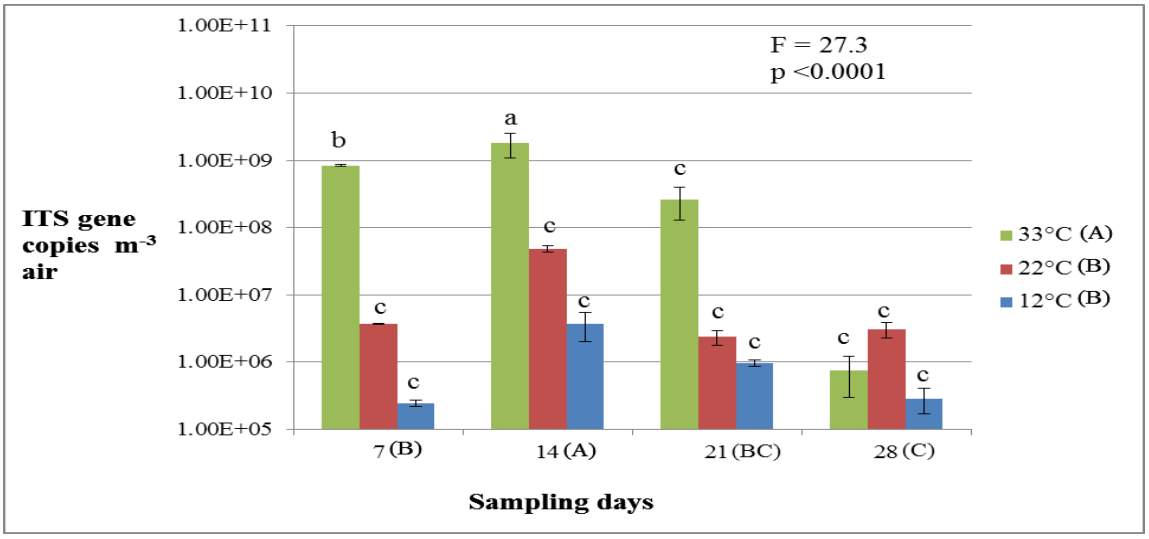

Figure 5.6 Abundance of *Aspergillus fumigatus* in bioaerosols emitted from compost at different temperatures. 7 = day 7; 14 = day 14; 21 = day 21 and 28 = day 28 of the composting experiment. Error bars represent standard error of mean (*n*=3). Significant differences are shown by ANOVA and Tukey's HSD. Different letters in brackets after temperature, sampling days or above bars indicate significant differences at 0.05 confidence interval.

5.2.5 Effect of temperature on fungal communities in bioaerosols from compost

The DGGE profile of fungal ribosomal DNA PCR amplicons from bioaerosols at 33°C (fig 5.7a) indicates similar band profile distribution in all the samples i.e. "N1-a". Some fingerprints observed on day 7 disappeared in the other weeks (days14, 21 and 28) (N2-a-N2-b). One of the samples of day 14 (2^{nd} – c) indicated lesser fingerprint incidence compared to the other two samples. Similarly some fingerprints appeared in day 21 and 28 were different from days 7 and 14 (N3-a-N3-b); whereas, "N4-a – N4-b" represents fingerprints only present in day 28.

Cluster analysis of the banding profile of fungal communities revealed almost 85% similarity between days 21 and 28; whereas, 70% similarity was recorded among days 7 and 14 days. Overall, 50- 40% similarity was recorded between the clusters of day 7 and 14 and the clusters of days 21 and 28 (Fig 5.7b)

DGGE banding profiles of fungal PCR amplicons obtained at 22°C indicated the abundance of some fingerprints in all of the samples (P1-a); whereas, higher band profile structure was observed in day 14 (P2-a – P2-b). A decrease in fingerprint distribution was detected in days 21 and 28 as faint bands (P3-a- P3-b) show the presence of some species in days 21 and 28 (fig 5.8a).

Cluster analysis indicated 70% similarity between the fingerprints of days 7 and14; whereas, almost 80% similarity was recorded between days 21 and 28. Overall, 40% similarity was found between the clusters of the days seven and fourteen and the clusters of days 21 and 28 (Fig 5.8b).

DGGE banding patterns of fungal PCR amplicons of bioaerosol collected on different days at 12°C (fig 5.9a) showed the presence of some fingerprints in day 7 which disappeared by day 14 (R2-a). Similar fingerprint distribution was also observed in all the samples (R1-a). While "R3-a" represents abundant fingerprint on day 14, "R4-a" indicates fingerprint richness on days 21 and 28. Community structure indicated that fingerprints appeared in day

seven and fourteen (R5-a) vanished by days 21 and 28; whereas cluster analysis showed almost 70% similarity between the fingerprint structure of days 7 and 14; however, almost 80% similarity was recorded on days 21 and 28. Overall, about 60% similarity was recorded between the clusters of days 7 and 14 and the clusters of days 21 and 28 (Fig 5.9b).

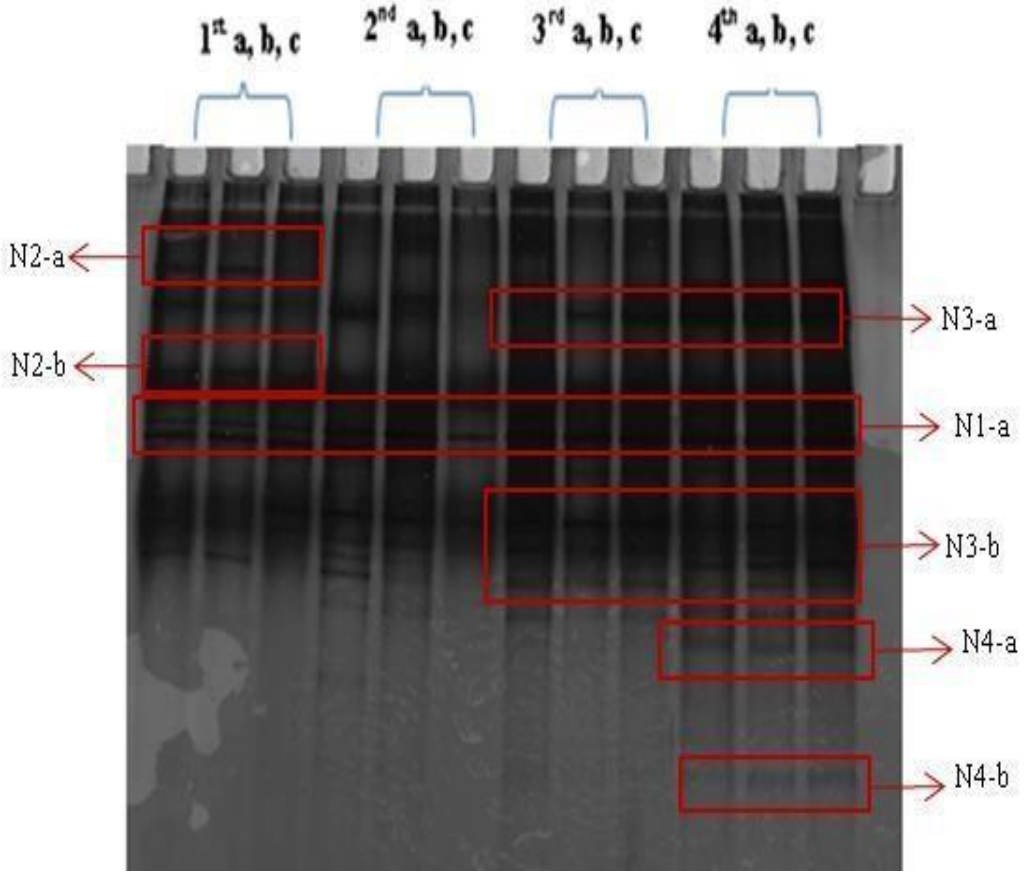

Figure 5.7 (a) DGGE profiles fungal ribosomal DNA (ITS2 region) PCR amplicons of bioaerosols from the compost box at 33°C.

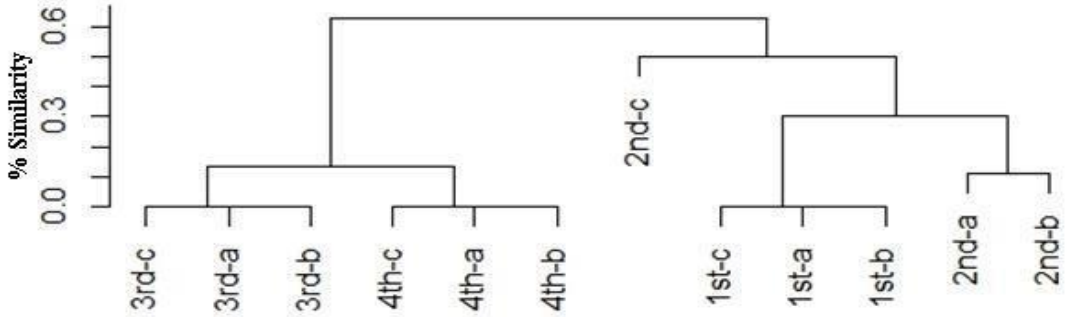

Figure 5.7 (b) Cluster dendrogram of the banding pattern in figure 5.7 (a) using a complete linkage (Jaccard cofficient). 1st a, b, c = day 7; 2nd a, b, c = day 14; 3rd a, b, c = day 21; 4th a, b, c = day 28.

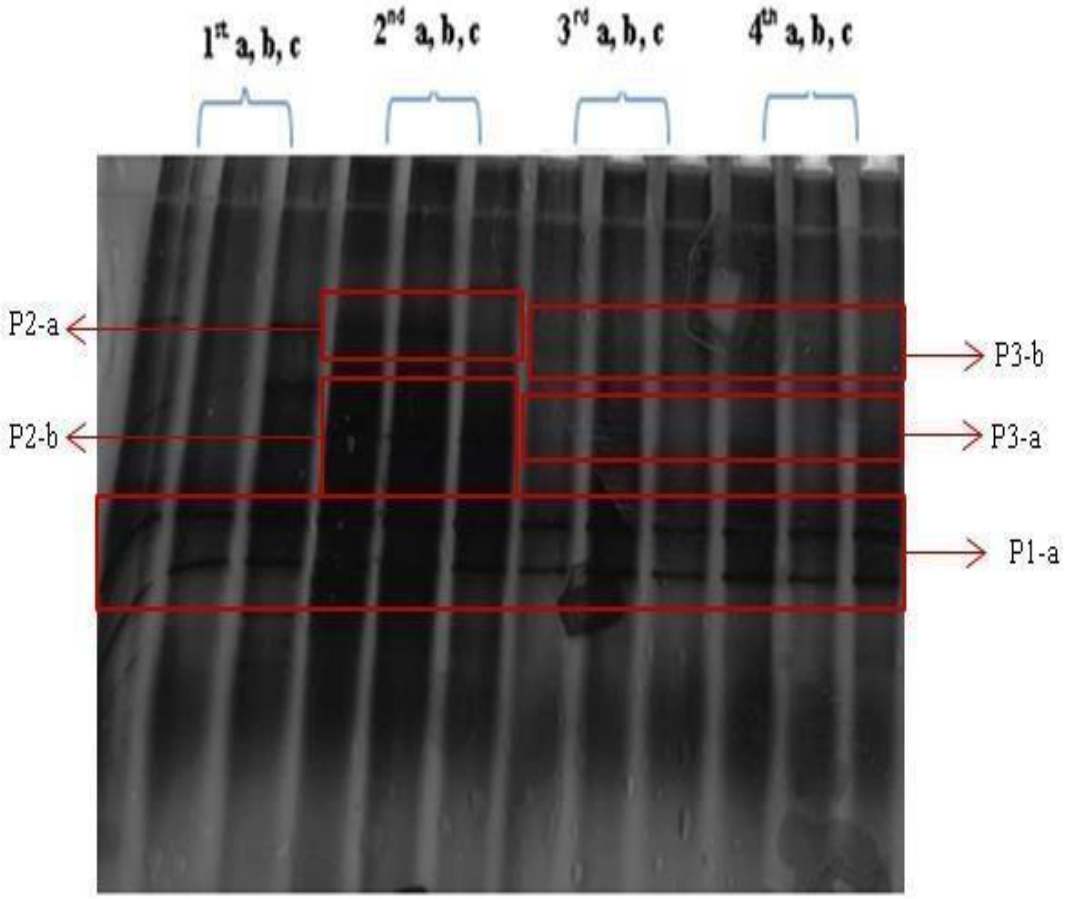

Figure 5.8 (a) DGGE profiles fungal ribosomal DNA (ITS2 region) PCR amplicons of bioaerosols from the compost box at 22°C.

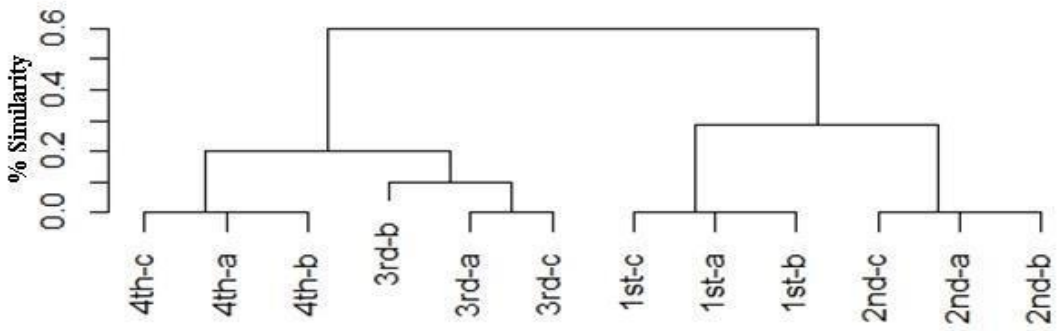

Figure 5.8 (b) Cluster dendrogram of the banding pattern in figure 5.8 (a) using a complete linkage (Jaccard cofficient). 1st a, b, c = day 7; 2nd a, b, c = day 14; 3rd a, b, c = day 21; 4th a, b, c = day 28.

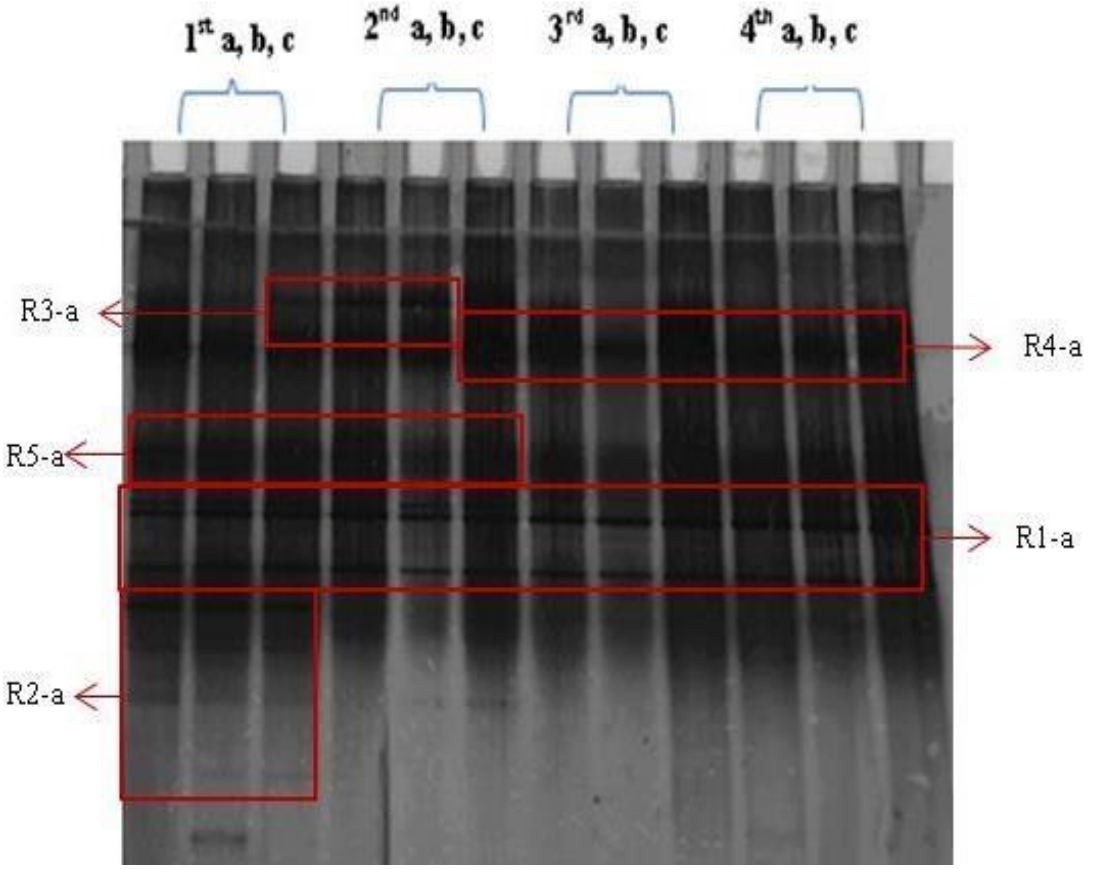

Figure 5.9 (a) DGGE profiles fungal ribosomal DNA (ITS2 region) PCR amplicons of bioaerosols from the compost box at 12°C.

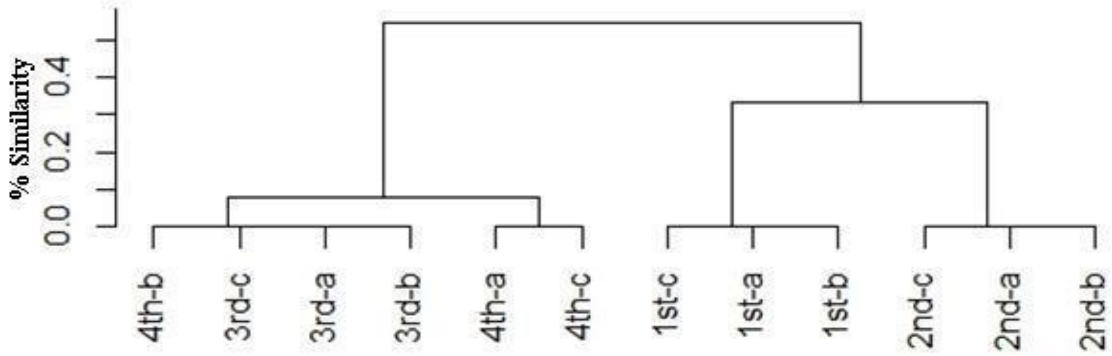

Figure 5.9 (b) Cluster dendrogram of the banding pattern in figure 5.9 (a) using a complete linkage (Jaccard cofficient). 1st a, b, c = day 7; 2nd a, b, c = day 14; 3rd a, b, c = day 21; 4th a, b, c = day 28.

5.2.6 Effect of moisture content on bacterial abundance in bioaerosols from compost

The abundance of bacteria in bioaerosols released from 40% compost recorded on day 7 and day 14 decreased to one order of magnitude by day 21 that then decreased further to one order of magnitude by day 28 (Table 5.5, Fig 5.10). Humidity increased continuously throughout the experiment from 16% in the first week to 88% by week four (Table 5.1). The abundance of bacteria obtained in bioaerosols from 60% compost on the day seven increased to three orders of magnitude by day fourteen that decreased to one order of magnitude from day 14 to day 21 and day 21 to day 28 (Table 5.5, Fig 5.10). Humidity inside the compost box increased from 22% in the first week to 94% by week four (Table 5.1). Bioaerosol samples collected in the first week of the 80% compost experiment did not produce results during amplification. The abundance of bacteria obtained in the second week decline to two orders of magnitude by day 21, followed by a further decline of two orders of magnitude by day 28 (Table 5.5, Fig 5.10). Humidity increased continuously throughout the experiment from 28% in week one to 97% in week four (Table 5.1).

Table 5.5 Quantification of bacteria in bioaerosols emitted from compost with different moisture contents

Sampling days	40%	60%	80%
7	7.9×10^9	7.8×10^7	
14	2.03×10^9	4.9×10^{10}	4.4×10^8
21	2.6×10^8	8.4×10^9	1.3×10^6
28	8.3×10^7	1.2×10^8	9.1×10^4

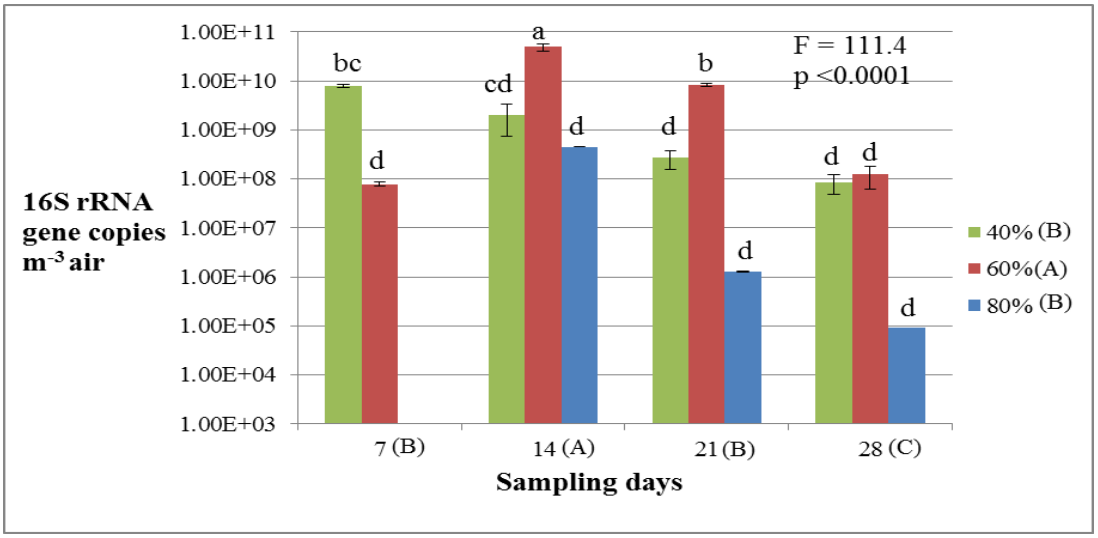

Figure 5.10 Abundance of bacteria in bioaerosols emitted from compost with different moisture contents. 7 = day 7; 14 = day 14; 21 = day 21 and 28 = day 28 of the composting experiment. Error bars represent standard error of mean (*n*=3).). Significant differences are shown by ANOVA and Tukey's HSD. Different letters in brackets after moisture content, sampling days or above bars indicate significant differences at 0.05 confidence interval.

5.2.7 Effect of moisture content on abundance of *Saccharopolyspora rectivirgula* in bioaerosols from compost

Saccharopolyspora rectivirgula, initial abundance in bioaerosols from compost with 40% moisture level indicated an increase of one order of magnitude from day 7 to day 14 that decreased to one order of magnitude by day 21 till day 28 (Table 5.6;Fig 5.11). *Saccharopolyspora rectivirgula*, abundance from compost with 60% moisture level increased one order of magnitude by day 14. However, a decrease in the abundances of one order of magnitude was observed from day 21 to day 28. In the compost box with 80% moisture level *Saccharopolyspora rectivirgula*, abundances declined one order of magnitude from day 21 till day 28 (Table 5.6;Fig 5.11)

Table 5.6 Quantification of *S. rectivirgula* in bioaerosols emitted from compost with different moisture contents

Sampling days	40%	60%	80%
7	8.2×10^6	8.9×10^6	
14	6.9×10^7	7.3×10^7	8.3×10^5
21	3.5×10^6	3.5×10^7	1.7×10^5
28	3.3×10^6	6.5×10^6	1.5×10^4

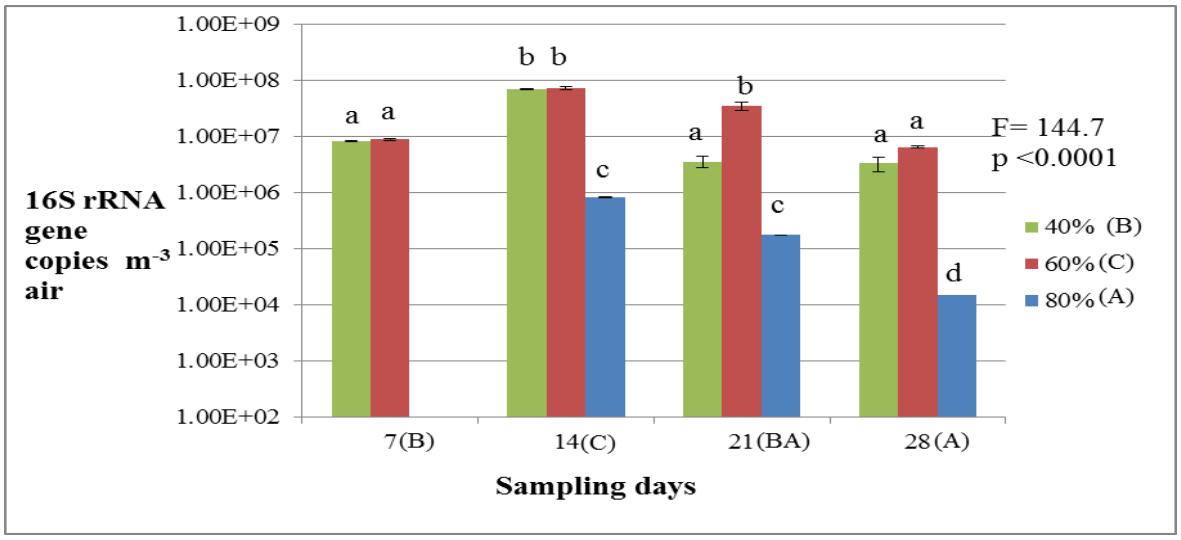

Figure 5.11 Abundance of *S. rectivirgula* in bioaerosols emitted from compost with different moisture contents. 7 = day 7; 14 = day 14; 21 = day 21 and 28 = day 28 of the composting experiment. Error bars represent standard error of mean (*n*=3). Significant differences are shown by ANOVA and Tukey's HSD. Different letters in brackets after moisture content, sampling days or above bars indicate significant differences at 0.05 confidence interval.

5.2.8 Effect of moisture content on bacterial community diversity in bioaerosols from compost.

DGGE patterns of bacterial 16S rDNA PCR amplicons of bioaerosols from compost with 40% moisture level indicated low fingerprint distribution on day 7; whereas, higher fingerprint distribution was recorded in samples collected on day 14 and reduced in number on day 21 (S1-a – S1-c) . The banding arrangement showed a decrease in the number of band profiles in the samples taken on days 21 and 28 (Fig 5.12a). "S2-a" and "S4-a" represent the abundance of band profiles on days 14 and 21 respectively. Some fingerprints were represented across days 7, 14 and 21 (S3-a) whereas, the faint band indicates the presence of fingerprints on day 28 (S5-a). Hierarchal cluster analysis showed that the fingerprint structure of samples on day seven and fourteen were almost 50% similar with each other. The banding profile indicated that the cluster of day 28 shared almost 30% similarity with that of day 21. However, the fingerprint distribution on days 7 and 14 cluster showed indicated 20% similarity with the cluster of days 21 and 28 (Fig 5.12b).

In bioaerosols collected from compost with 60% moisture content, the bacterial community analysis from the DGGE profiles represented some of the fingerprints that appeared on days 7 and 14 (U3-a – U3-b) were not observed in the corresponding days and some of the fingerprints were rich on day 14 as indicated in "U1-a – U1-e"; however, some of the fingerprints (U2-a – U2-b) were present in all of the samples (Fig 5.13a).

However, the faint bands (U4-a – U4-c) indicates the presence of fingerprints on days 21 and 28. Hierarchal cluster analysis showed that the fingerprint structure of samples from days 7 and 14 were almost 45% similar with each other. The banding profile indicated that cluster of day 28 shared almost 40% similarity with that for day 21. However, the fingerprint distribution in the cluster of days 7 and 14 was diverse and indicated 20% similarity with days 21 and 28 (fig 5.13b). Bacterial community analysis from the DGGE profiles in bioaerosols from compost with 80% moisture level represented the presence of a diverse microbial population (fig 5.14a). Some of the fingerprints which were rich in days

14 and 21 reduced by day 28 (W2-a & W3-a – W3-b); however, some of the fingerprints were present in all samples (W1-a – W1-b). “W4-a – W4-d” represents the presence of fingerprints on day 28. Cluster analysis of the banding patterns indicated almost 40% similarity between days 21 and 28 which together shared almost 20% similarity with the fingerprint structure of day 14 (Fig 5.14b).

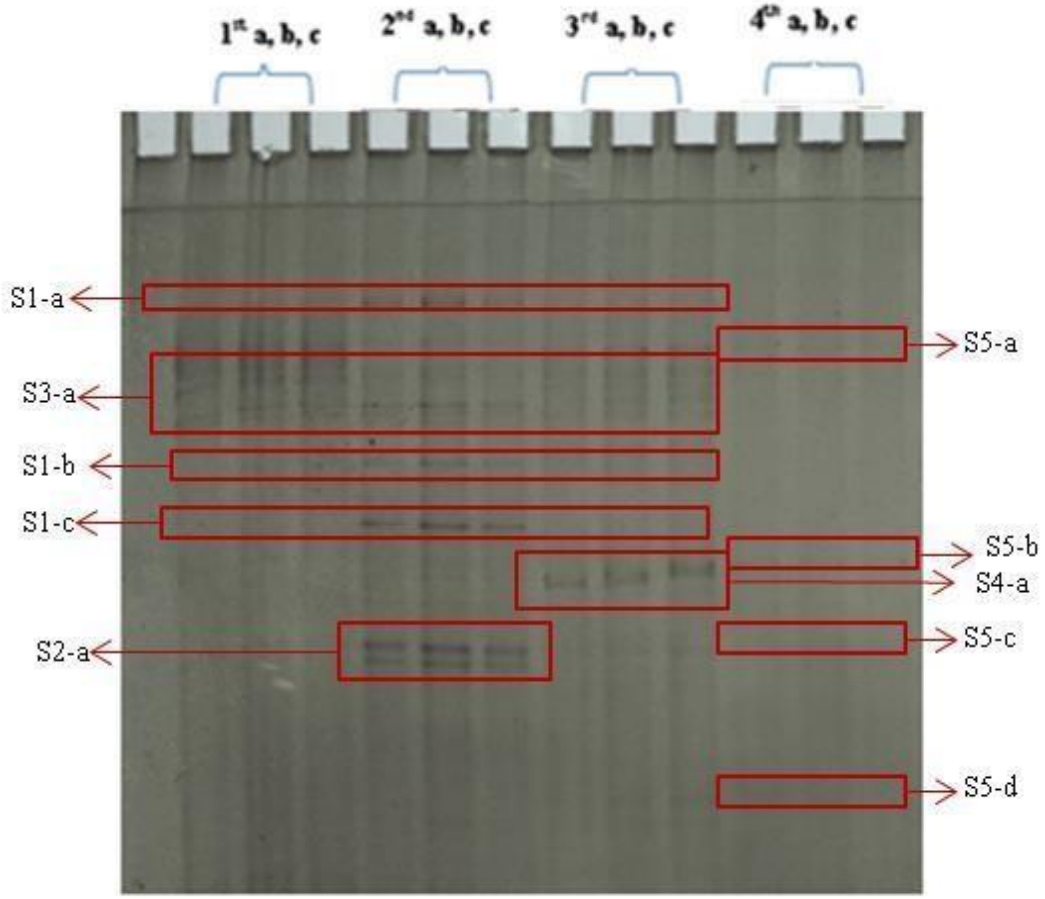

Figure 5.12 (a) DGGE profiles of bacterial 16S rDNA PCR amplicons of bioaerosols from compost with 40% moisture content.

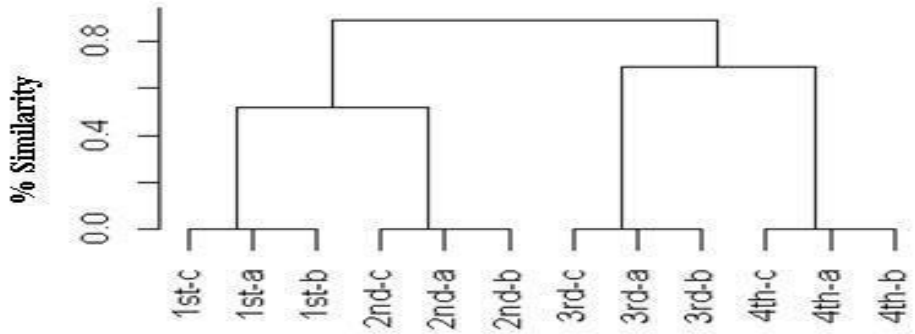

Figure 5.12(b) Cluster dendrogram of the banding pattern in figure 5.12(a) using a complete linkage (Jaccard cofficient). 1st a, b, c = day 7; 2nd a, b, c = day 14; 3rd a, b,c = day 21; 4th a, b, c = day 28.

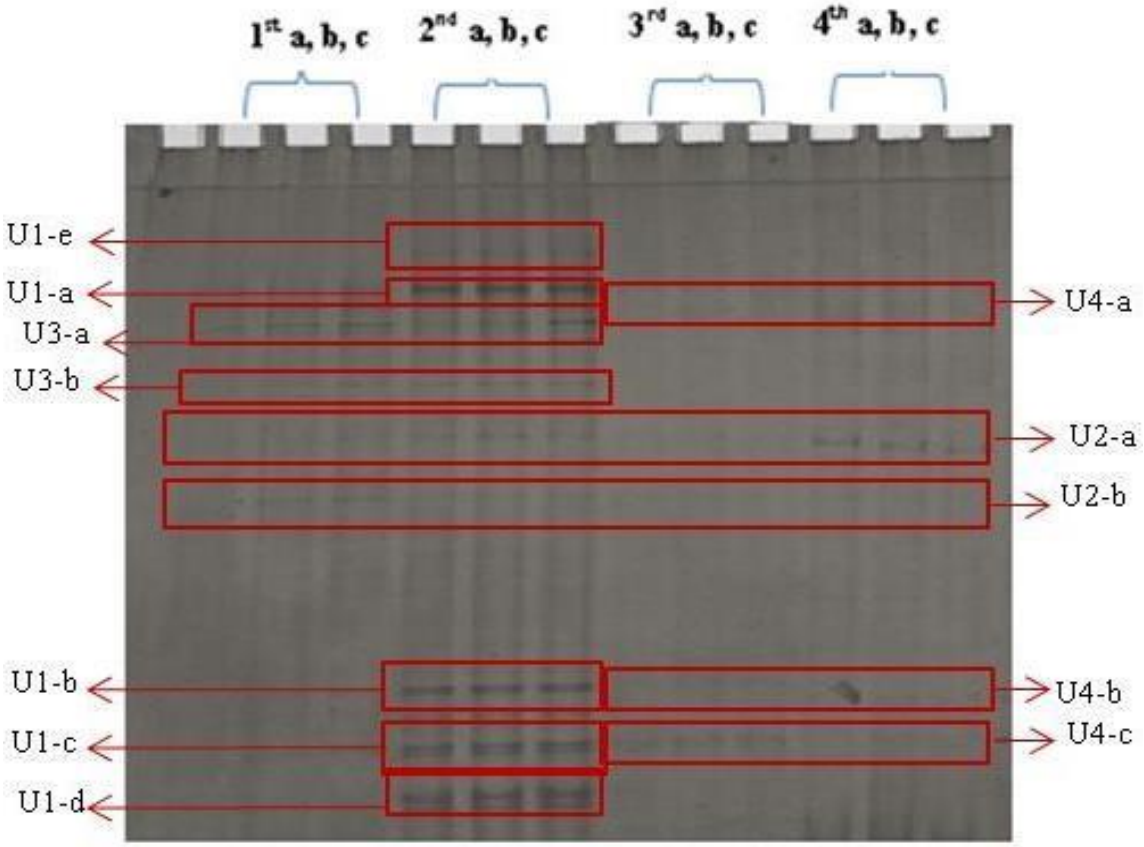

Figure 5.13(a) DGGE profiles of bacterial 16S rDNA PCR amplicons of bioaerosols from compost with 60% moisture content.

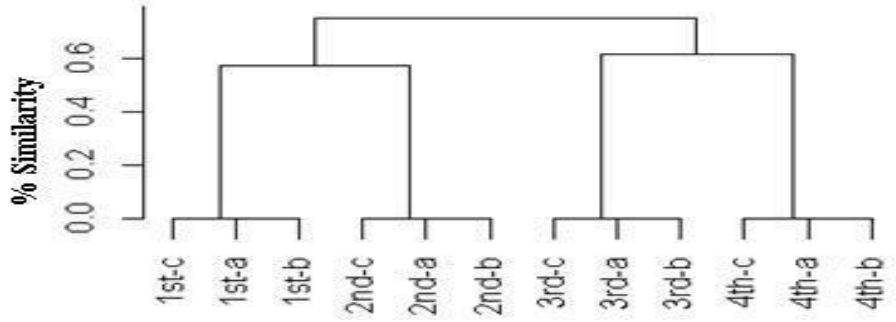

Figure 5.13(b) Cluster dendrogram of the banding pattern in figure 5.13(a) using a complete linkage (Jaccard cofficient). 1st a, b, c = day 7; 2nd a, b, c = day 14; 3rd a, b,c = day 21; 4th a, b, c = day 28.

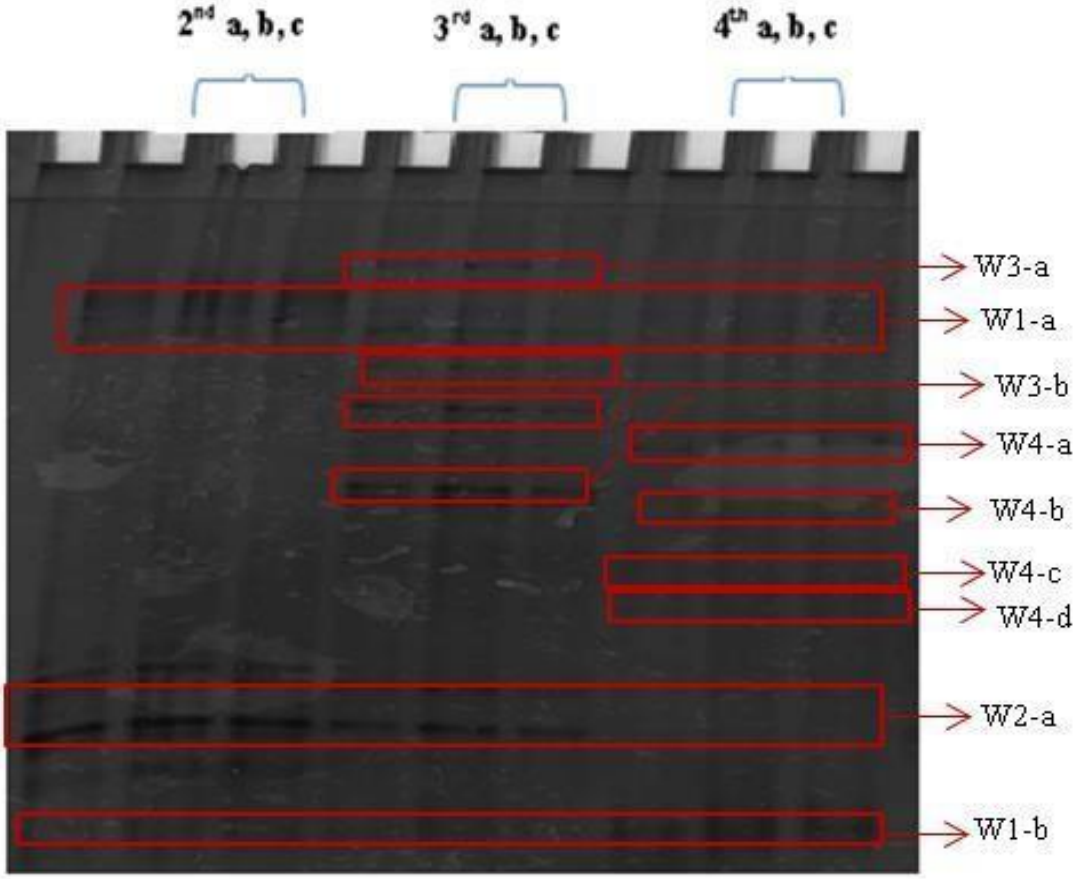

Figure 5.14(a) DGGE profiles of bacterial 16S rDNA PCR amplicons of bioaerosols from compost with 80% moisture content.

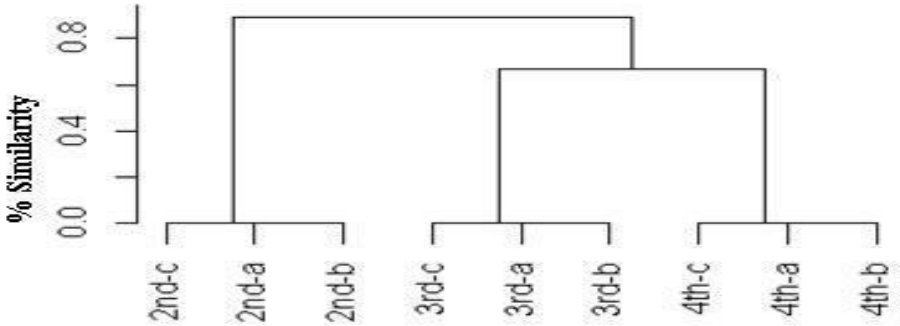

Figure 5.14(b) Cluster dendrogram of the banding pattern in figure 5.14(a) using a complete linkage (Jaccard cofficient). 2nd a, b, c = day 14; 3rd a, b, c = day 21; 4th a, b, c = day 28.

5.2.9 Effect of moisture content on abundance of *Aspergillus fumigatus* in bioaerosols from compost

The abundance of *Aspergillus fumigatus,* in bioaerosols from compost with 40% moisture level indicated an increase of one order of magnitude from week one (day7) to week two (day 14). The abundance began to decrease gradually to one order of magnitude in week three (day 21) and this continued through to week four (day 28) (Table 5.7, Fig 5.15). The abundance of *Aspergillus fumigatus* in bioaerosols obtained from 60% compost increased by one order of magnitude from week one (day seven) to week two (day 14). A decline of one order of magnitude was observed between day 21 and day 28 (Table 5.7, Fig 5.15). With 80% moisture levels of the compost the abundances of *Aspergillus fumigatus* indicated decline of one order of magnitude from day 14 to day 21 and decreased further to one order of magnitude in week four (day 28) (Table 5.7, Fig 5.15).

Table 5.7 Quantification of *Aspergillus fumigatus* in bioaerosols emitted from compost with different moisture contents.

Sampling days	40%	60%	80%
7	2.4×10^5	5.2×10^5	
14	3.8×10^6	3.1×10^6	8.3×10^6
21	9.2×10^5	2.4×10^6	2.8×10^5
28	2.8×10^5	2.6×10^5	2.4×10^4

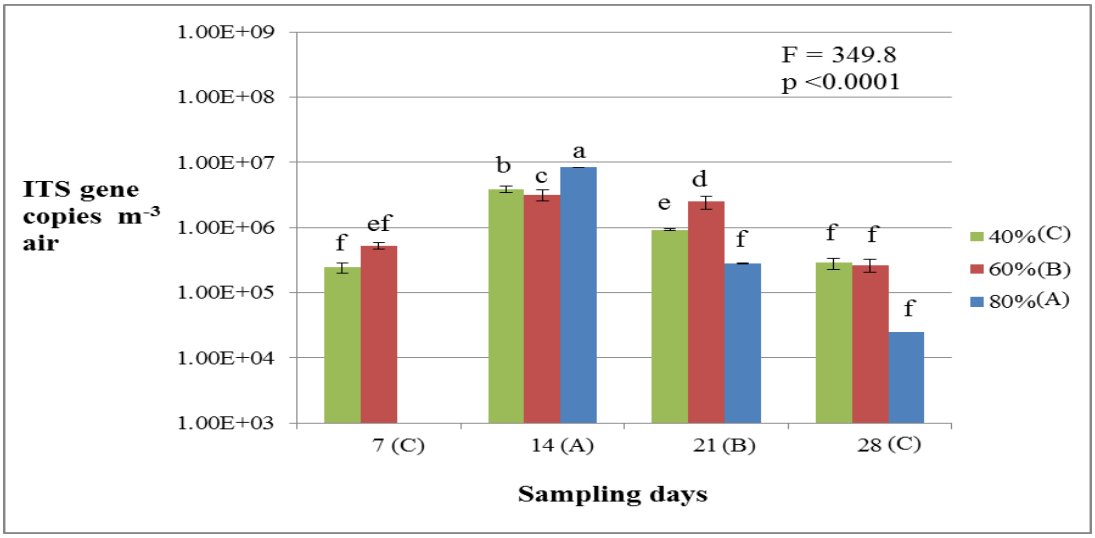

Figure 5.15 Abundance of *Aspergillus fumigatus* in bioaerosols emitted from compost with different moisture contents. 7 = day 7; 14 = day 14; 21 = day 21 and 28 = day 28 of the composting experiment. Error bars represent standard error of mean (*n*=3). Significant differences are shown by ANOVA and Tukey's HSD. Different letters in brackets after moisture content, sampling days or above bars indicate significant differences at 0.05 confidence interval.

5.2.10 Fungal communities' analysis in bioaerosols from compost with different moisture content

DGGE profile of fungal ribosomal DNA PCR amplicons of bioaerosols from compost with 40% moisture level indicated a similar fingerprint distribution and abundance of some fingerprints in all the samples (T1-a) (Fig 5.16a). Some band profiles abundant on days 7 and 14 were reduced on day 21 and increased again on day 28 (T2-a). "T3-a –T3- b" represent fingerprints that were rich on days 7 and 14 decreased on days 21 and 28 while "T4-a" indicates less number of band profiles in the weeks. Cluster analysis of the banding profile revealed almost 30% similarity between the samples on days 21 and 28 (Fig 5.16b). Overall, 20% similarity was recorded between the cluster of seven and fourteen days and the cluster of days 21 and 28.

DGGE banding patterns of fungal PCR amplicons of bioaerosols collected on different weeks from compost with 60% moisture content showed the presence of some fingerprints in days 7 and 14 (V2-a) disappeared in the following days. Similar fingerprints were also observed in all the samples (V1-a – V1-c). "V3-a" and "V4-a – V4- b" specifies the abundance of band profiles on days 21 and 28 respectively (Fig 5.17a). Cluster analysis indicated 80% similarity between the fingerprints of days 7 and 28; whereas, almost 65% similarity was recorded between the fingerprints of day 28 and those on days 14 and 21. Overall, 30% similarity was found between the clusters of day seven and that of the other days, indicating a diverse banding profile in the day seven of the experiment (Fig 5.17b)

DGGE banding patterns of fungal PCR amplicons of bioaerosols (Fig 5.18a) collected on different weeks from compost with 80% moisture content indicated abundance of some similar band profiles in all the samples (X1-a – X1-c); whereas, "X2-a – X2-c" shows fingerprints that were found in all samples however, faintness of bands indicates less abundance of the fingerprints. "X3-a" indicates species that only appeared on day 28. Cluster analysis (Fig 5.18b) showed almost 50% similarities between the microbial structure on days 21 and 28; however, almost 20% similarity was recorded between the cluster of day 14 and that of days 21 and 28.

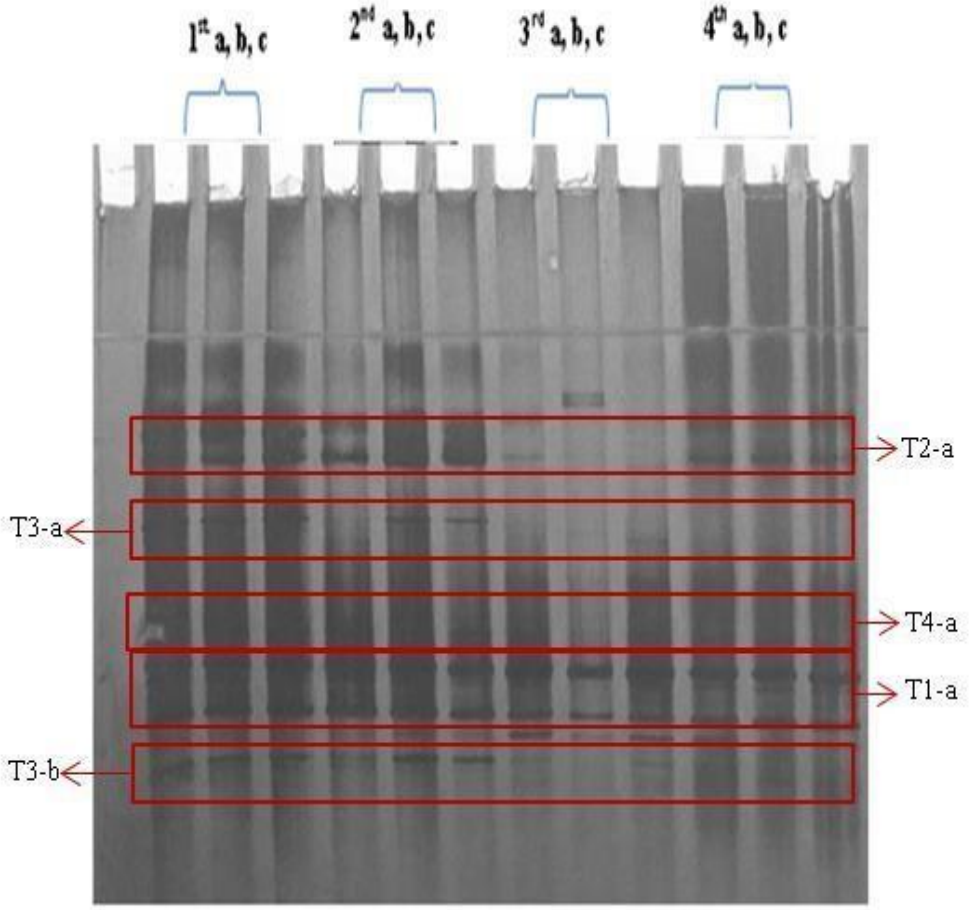

Figure 5.16 (a) DGGE profiles fungal ribosomal DNA (ITS2 region) PCR amplicons of bioaerosols from compost with 40% moisture content.

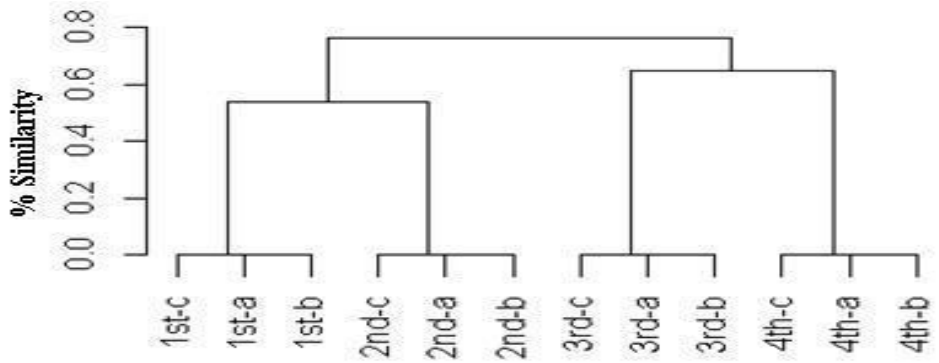

Figure 5.16(b) Cluster dendrogram of the banding pattern in figure 5.16(a) using a complete linkage (Jaccard cofficient). 1st a, b, c = day 7; 2nd a, b, c = day 14; 3rd a, b, c = day 21; 4th a, b, c = day 28.

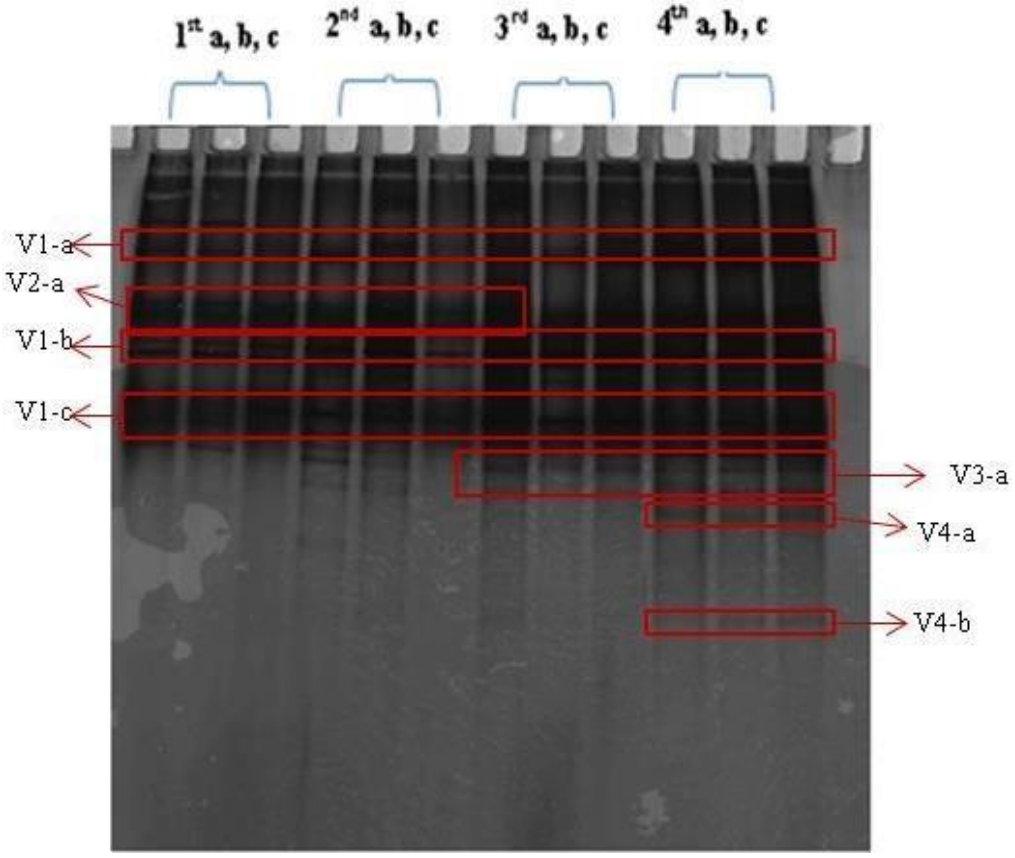

Figure 5.17(a) DGGE profiles fungal ribosomal DNA (ITS2 region) PCR amplicons of bioaerosols from compost with 60% moisture content.

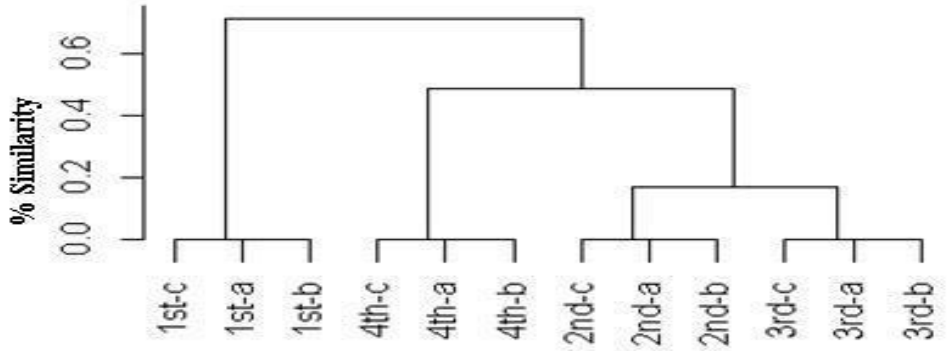

Figure 5.17(b) Cluster dendrogram of the banding pattern in figure 5.17(a) using a complete linkage (Jaccard cofficient). 1st a, b, c = day 7; 2nd a, b, c = day 14; 3rd a, b, c = day 21; 4th a, b, c = day 28.

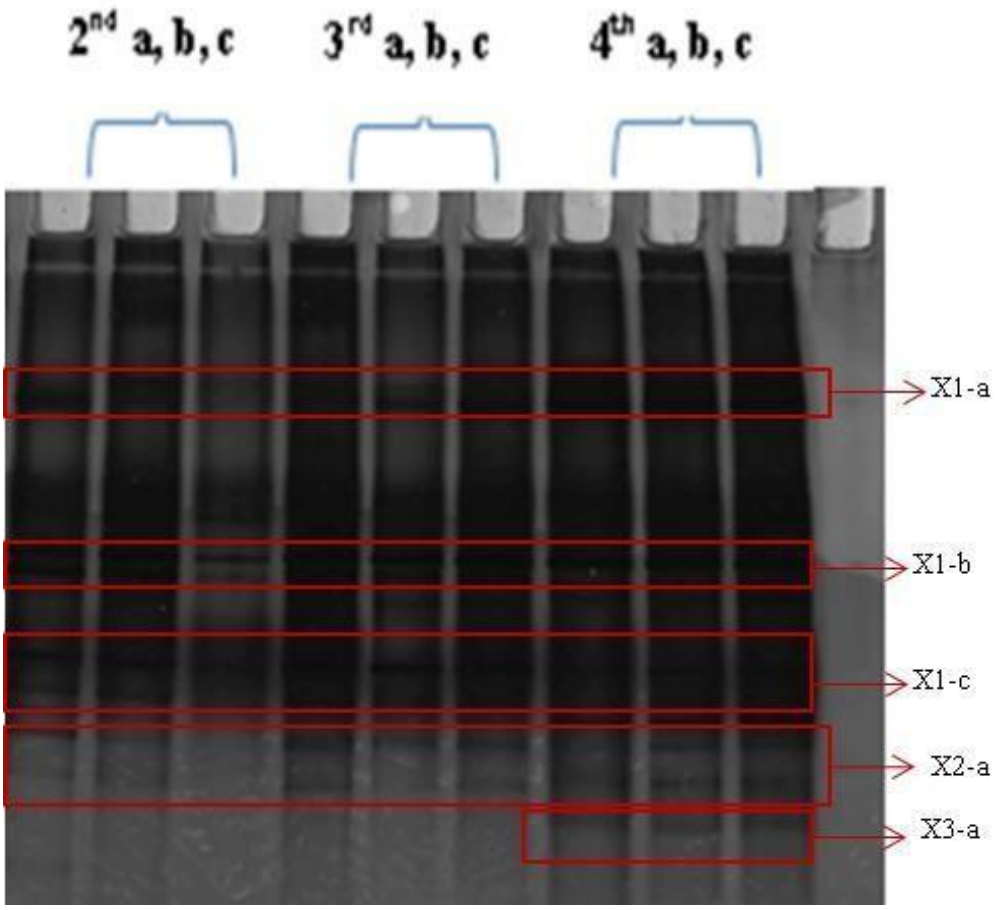

Figure 5.18(a) DGGE profiles fungal ribosomal DNA (ITS2 region) PCR amplicons of bioaerosols from compost with 80% moisture content.

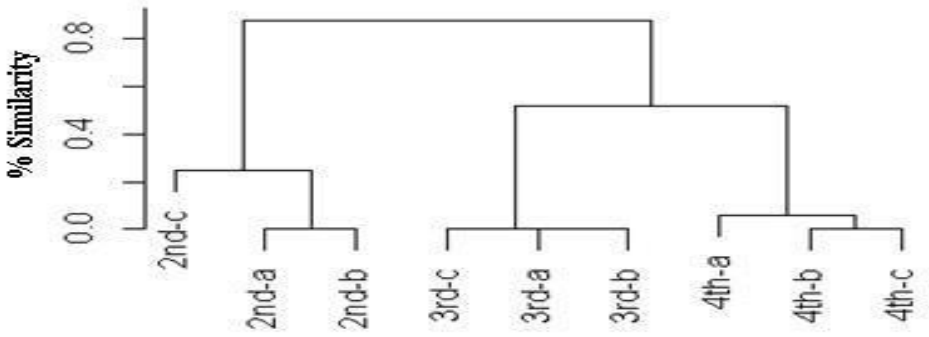

Figure 5.18(b) Cluster dendrogram of the banding pattern in figure 5.18(a) using a complete linkage (Jaccard cofficient). 2nd a, b, c = day 14; 3rd a, b, c = day 21; 4th a, b, c = day 28.

5.3 Discussion

This study was conducted with a key aim to assess the impact of temperature and moisture content on the microbial community composition and abundance of bioaerosols from compost under laboratory conditions. Previous field based studies have suggested the influence of meteorological parameters (temperature, humidity and wind speed) on the composition and emission of bioaerosols from composting sites (Wery, 2014). However, there has been uncertainty about the relationship and impact of individual factors on the emission and microbial diversity of bioaerosols from the composting process. Therefore, the objective of this study was to evaluate the effect of different temperatures on the concentration and composition of bioaerosols emitted from compost.

Temperature is the key factor in the composting environment and it determines the stability of the organic matter and affects the microbial activity during the composting process (Fogarty & Tuovinen, 1991). Depending on temperature, microbes in the process of composting are classified as mesophilic (<45°C) and thermophilic (45°C to 60°C) microorganisms. An increase in the temperature above 60°C impedes the activity of the mesophilic microbes. It has been suggested that an increase in the temperature during composting is mainly due to the biological activity of the microorganisms resulting in the generation of heat and subsequent rise in the temperature of the pile (Bertoldi *et al.,* 1983). In a study, Nakasaki *et al.,* (1985) indicated >60°C as the optimum temperature for the microbial activity. Other studies have also confirmed the optimum temperature to be below 60°C due to the decrease in microbial community diversity and biomass as result of high temperatures (McKinley *et al.,* 1984;).

The presence of moisture supports the microbial activity in the compost and leads to the decomposition of the organic matter. The moisture content of the composting process is essential in transporting dissolved nutrients to the microorganisms for their metabolic activities (Liang et al., 2003). The optimum moisture content is dependent on the type and

physiochemical features of the waste material. Various studies have employed a range of moisture contents i.e. 50% - 70% during different composting systems (Tiquia *et al.,* 1996; Kalyuzhnyi *et al.,* 1999; Liang *et al.,* 2003; Guo *et al.,* 2012). Similarly in a laboratory scale experiment Kumar et al. (2010) reported 60% optimum moisture content during co-composting of green and food waste.

It was anticipated that considering each composting system has a diverse microbial community composition and abundance, variations in microbial diversity of the bioaerosols would occur during different time periods at different temperatures and with different moisture contents. Also higher microbial abundance was anticipated in bioaerosols emitted from compost at 33°C with a 60% moisture level. Q-PCR was employed to quantify the microbial abundance of bioaerosols and results indicated that lower concentrations of bacteria, *Saccharopolyspora rectivirgula* and *Aspergillus fumigatus* were obtained in the first week at different temperatures i.e. 33°C, 22°C and 12°C; however, an increase in the concentration was observed in the second week and afterwards a decline in the concentration was observed till week four of the experiment. However, a declining pattern in the bacterial abundance was observed at 12°C whereby the abundance decreased throughout the experiment. Maximum abundance of bacteria was obtained at 33°C as compared with 22°C and 12°C and, maximum abundance of *Saccharopolyspora rectivirgula* was observed in the second week of the 22°C experiment and minimum abundance was recorded at 12°C. Similarly, the concentration of *Aspergillus fumigatus* was a maximum at 33°C and minimum at 12°C. The higher abundance of microbes at 33°C indicates increased microbial activity as compared with lower temperatures. Miyatake *et al.* (2005) investigated the effect of high temperatures (54°C, 60°C, 63°C, 66°C and 70°C), on the species diversity of the compost, and suggested higher microbial activity at 54°C.

The lower abundances of microbes in the bioaerosols during the first week suggest lesser microbial activity in the compost, whereas the higher abundance during the second week suggests an increase in microbial activity of the compost as compared to later weeks. It has also been previously suggested that the increase in abundance can be possibly due to the

thermophilic stage in the composting system (Ishii *et al.*, 2000). Although 60% moisture content of the compost was adjusted before each experiment, the significant increase in the microbial abundance particularly during second week of the 33°C and 22°C may possibly be due to the increase in the microbial activity of the compost between 34% - 45% humidity. However, a reduction in the concentration was observed during weeks three and four and this may be due to the high humidity (64% - 93%) as well as drying of the compost as this can impede the microbial activity thus negatively influencing the bioaerosols emissions (Lighthart & Mohr, 1987). The higher abundance of bacteria during the first week at 12°C can be possibly due the rapid microbial activity in the mesophilic phase or early emergence of the thermophilic stage in the composting system as different physio- chemical parameters affect the microbial activity (Steger *et al*., 2005; Zhang *et al.*, 2016).

Quantification results of the influence of moisture levels on microbial abundance showed that although bacterial abundance was higher in the first week of 40% moisture content as compared with 60% moisture content, abundance decreased from the first to fourth weeks. Abundance recorded at 60% moisture content indicated an increase by the second week and overall higher abundance was obtained with 60% moisture content; whereas, abundance recorded at 80% moisture content indicated a declining pattern in abundance from the second to fourth weeks. The increased microbial abundance at 60% is similar with the results of previous studies that reported 60% as the optimal moisture level for microbial decomposition of the organic matter. In addition Suler and Finstein (1977) reported maximum microbial activity at 56°C to 60°C with 60% moisture content.

In contrast to bacteria, *Saccharopolyspora rectivirgula* abundance recorded at 40% moisture content showed a different pattern to bacterial abundance as it increased by the second week and then decreased in concentration till week four. Abundance recorded in 60% moisture content indicated a similar pattern of increase in the second week and then decline in concentration, however, abundance at 80% indicated a decrease in abundance from the second to fourth week. The concentration of *Aspergillus fumigatus* was higher in the second week at 40%, 60% and 80% moisture content and overall higher abundance of *Aspergillus*

fumigatus was recorded in the second week with 80% moisture content as compared with 40% and 60% moisture contents. However, with 80% moisture content, the sampling in week one did not generate any results for analysis (PCR), which is expected due to the high moisture content that may possibly have hampered the generation of bioaerosols. It has been suggested that higher moisture levels during composting results in decreasing oxygen availability by filling the pores between the particles (Miller 1991; Richard et al., 2002). In a study Tiquia *et al.* (1996) while indicating slow decomposition of compost at a high moisture level (70%), reported that higher moisture content (70%) resulted in a decreased microbial diversity as increased moisture levels restrict the utilisation of oxygen by microbes by influencing the exchange of gases and thus preventing the process of diffusion. In another laboratory based study Liang *et al.* (2003), while assessing the influence of temperatures and moisture content on the composting process, suggested that moisture content effects are more influential than temperature.

In order to evaluate the microbial diversity of the bioaerosols emitted from the compost during a laboratory scale experiment, denaturing gradient gel electrophoresis (DGGE) analysis of the amplicons produced from polymerase chain reaction was employed (Muyzer *et al.,* 1993). DGGE analysis revealed higher bacterial and fungal diversity during the second week of the sampling at 33°C and 22°C; however, higher bacterial and fungal population was observed during the first week at 12°C. Overall, higher microbial diversity observed at higher temperatures (33°C) can be justified as Tong & Lighthart (2000) and Jones & Cookson (1983) have observed higher bacterial and fungal concentrations during the warm season. Further, higher concentrations of bioaerosols at high temperatures can be justified in that the concentrations of bioaerosols increase during warm seasons and a positive relationship exists between temperature and the survival of the microbial flora of bioaerosols (Nielsen *et al.,* 1997; Schlosser *et al.,* 2009; Lin & Li 2000). However, a higher concentration of *S. rectivirgula* was obtained at 22°C and 12°C as compared with 33°C. This suggests that higher temperatures affect the concentration of *S. rectivirgula*. The microbial community composition analysis indicated the bacterial and fungal populations in

the bioaerosols from the compost continued to change during different weeks. Fingerprint results shown greater bacterial and fungal diversity during the second week at 40% and 60% moisture; however, higher microbial diversity was observed from compost adjusted with 60% moisture whereas a low community composition was recorded for 80% moisture content. Previously, it has been mentioned that higher moisture content can affect microbial activity i.e. filling the air spaces between the particles leading to the anaerobic conditions (Tiquia *et al.,* 1996), which could possibly hinder the generation of the bioaerosols.

Moreover, low moisture content affects the movement of microbes within the compost (Miller *et al*., 1989) and similarly Debertoldi *et al.* (1983) found that low levels of moisture lead to the desiccation and ultimately halting the composting process. Other studies (Suler & Finstein, 1977; McKinley & Vestal, 1984) indicated that optimum moisture content ranged between 50-60%. Tiquia *et al*. (1996) showed that an increase in moisture levels up to 70% reduces the microbial activity in composting systems. This discrepancy in optimum moisture level may be due to the variations between different compost substrates and different times of the composting process. Additionally, operating outside the optimum range of moisture content had a considerable adverse effect on the microbial activity and will slow down or terminate the composting process (Richard *et al.,* 2002). Previously it has been suggested that a positive relationship follows between temperature and ambient bioaerosols; whereas humidity showed a negative relationship with ambient bioaerosols (Grinn-Gofroń and Strzelczak, 2008).

5.4 Conclusions:

Results accomplished during this study suggest that variations in microbial community composition and abundance were observed at different temperatures and moisture levels during different days of the laboratory scale composting process. The presence of diverse microbial flora analysed through DGGE is an indication of the influence of temperatures and moisture contents on communities' composition of bacterial and fungal populations. Results showed that an increase in temperature (33°C) with 60% moisture levels leads to an increase in the abundance of bacterial and *A. fumigatus*, particularly in the second and third weeks of the composting; which also suggests that higher temperature increases the microbial abundance. However, higher temperature (33°C) has a negative effect on the abundance of *S. rectivirgula.* Overall, higher abundances at 60% moisture contents suggested it to be ideal for the composting process.

Chapter 6 Overall discussion

6.1 Concluding remarks and conclusion of concluding remarks

The dispersal of bioaerosols from the composting facilities is considered to have adverse health impacts, such as invasive aspergillosis, allergic rhinitis, allergic asthma, chronic bronchitis, extrinsic allergic alveolitis and toxic pneumonitis on the workers and residents nearby these facilities and this warrants assessment of their microbial composition and abundance (Taha *et al*. 2006; Walser *et al.*, 2015). In terms of the extent of dispersal; both in the UK and Pakistan's composting facilities, the results indicated higher concentrations of bioaerosols onsite which are possibly due to the composting activities, thus generating higher emissions (Taha *et al.*, 2007; Wery, 2014).

The overall aims of the present study were to investigate the microbial composition and abundance of bioaerosols from composting facilities and assess the influence of temperature and moisture levels on the microbial diversity of bioaerosols and abundnace. It was hypothesized that the dispersal of bioaerosols from composting facilities would decrease with increasing distances from the point source. The results obtained from the UK and Pakistan's composting sites suggested maximum dispersal onsite and minimum upwind. It has been suggested through studies that onsite activities increase the emissions of bioaerosols and this results in higher abundance nearby (Wéry, 2014; Recer *et al.*, 2001). Moreover, studies have indicated that site specific activities, typography and atmospheric conditions affect the microbial community composition and abundance of bioaerosols from composting sites (Swan *et al.*, 2003; Pankhurst *et al.*, 2011).

Seasonal meteorological influences are suggested to modify the concentration and composition of compost aerosols and studies have indicated higher microbial abundances during summer and autumn periods with a decline in winter (Nielsen et al,, 1995; Recer et al., 2001; Schlosser et al., 2009). Seasonal effects on the microbial abundance of bioaerosols released from the UK composting facility were also observed, whereby higher abundances

of bacteria and specifically the bacterial pathogen *S. rectivirgula* were found in the summer compared to the autumn and winter. Meteorological variables such as, temperature, humidity and ultraviolet radiation are suggested to influence the dispersal and initial release of bioaerosols (Jones & Harrison, 2004). The abundances attained during the seasonal sampling regimes revealed maximum emissions of bioaerosols during summer as compared with autumn and winter. The increased abundance in summer may be due to the low humid air that could aid in enhanced dispersal of bioaerosols as high humidity negatively influences emissions (Lighthart & Mohr, 1987). This can also be explained through the influence of wind speed which is a significant factor in the increased emissions, and hence higher concentrations of microbial flora. The wind speed at the Colchester composting site ranged from 3 to 5 m/s whereas, 0.2 to 0.5 m/s were recorded at the Pakistan sites. Further, wind speed was approximately the same in each sampling regime at the Colchester composting site, so the possible meteorological impact on the increased dispersion of aerosols may be due to temperature and humidity factors. An increase in atmospheric temperature reduces the humidity which results in the wide dispersion of particles.

In Pakistan's sampling campaign, higher microbial abundances were recorded onsite and a decrease in the abundance was observed with increasing distance across each composting facility. The presence of significant microbial flora at 250 m downwind locations may be possibly due to onsite activities. Pakistan composting sites had higher temperatures, lower humidity and little wind speed. The abundances of bioaerosols obtained from Pakistan's sites are comparatively less than that in Colchester. Although, previous studies have indicated that warmer temperatures enhance the emission of aerosols, this decrease in emission may possibly be due to little wind speed (0. the results suggest that the relationship between bioaerosol emissions and meteorological parameters is highly complex. It was anticipated that the considering hot atmospheric conditions of Pakistan, higher microbial abundance would occur compared to that in the UK.

DGGE fingerprint analysis indicated variations between bacterial and fungal populations of the compost as well as bioaerosols obtained from the UK composting site at different

locations during different seasons. These findings can also be evidenced from Pankhurst *et al.* (2012). It was also anticipated that fungal microbial populations would disperse furthest distances downwind of the site comparative to bacterial communities and DGEE analysis indicated this. The presence of fungal population at 250 m downwind relative to bacteria at the UK and Pakistan composting sites indicated the high dispersal of spores which can become easily airborne Fletcher *et al.* (2008) due to the drier conditions as is helps in the emissions of bioaerosols (Le Goff *et al*., 2010).

The individual impact of temperature and moisture content (humidity) indicated that temperature enhances the emission of bioaerosols with higher abundances at 33°C as compared with 22°C and 12°C. It has been reported in previous studies that higher bacterial and fungal abundances were observed during warm seasons (Tong & Lighthart, 2000; Jones & Cookson, 1983). The abundance of *A. fumigatus* during the laboratory scale composting was a maximum at a high temperature (33°C) as compared with 22°C and 12°C which suggests that warm temperatures enhance the abundance of *A. fumigatus*. The higher generation of bioaerosols at 33°C indicated intense microbial activity in the compost and subsequent releases resulted in higher microbial emissions. However, the decrease in the abundance of aerosols after two weeks is particularly due to an increase in the humidity inside the box which hampered the dispersal of bioaerosols.

Comparing the seasonal sampling data with the laboratory scale experiment, it justifies that that higher temperature results in the increased bacterial abundance. The maximum abundance of bacteria recorded at 33°C was 7.1 x 10^{10} 16S rRNA gene copies/m^3 which is approximately similar with the maximum abundance of bacteria recorded during summer i.e. 5.6 x 10^{10} 16S rRNA gene copies/m^3. However, the abundances of bacteria from Pakistan (37°C) composting facilities were less than this i.e. 6.8 x 10^8 16S rRNA gene copies/m^3 - 9.1 x 10^9 16S rRNA gene copies/m^3. This difference in abundance may be possibly due to other factors i.e. scale of composting activity, feedstock composition, onsite activities and climatic parameters (Swan *et al.*, 2003; Adams and Frostick, 2009; Wery,

2014). Similarly, the maximum abundance of *Saccharopolyspora rectivirgula* obtained at 22°C (8.7 x 10^8 16S rRNA gene copies/m^3) showed similarity with the abundance from the summer season (22.3°C) i.e. 8.8 x $10^8$16S rRNA gene copies/m^3. However, the maximum abundances of *Saccharopolyspora rectivirgula* obtained at Pakistan's (38°C) composting sites were 9.7 x 10^6 16S rRNA gene copies/m^3 - 2.7 x 10^7 16S rRNA gene copies/m^3 showed approximate similarity with abundance at 33°C i.e. 3.4 x 10^7 16S rRNA gene copies/m^3. This suggests that higher temperatures negatively influence the abundance of *S. rectivirgula*. Moreover, in ambient air, an increase in temperature is known to decrease humidity; however, in laboratory settings, an increase in temperature will dry out the compost which causes the humidity to increase. This would have a negative impact on the particles to become airborne, hence resulting in a decrease in concentration. Concerning the influence of moisture content, as mentioned in previous studies, high moisture content results in the filling of spaces between compost particles thus limiting microbial activity which promotes anaerobic conditions in the composting environment (Miller *et al*., 1989; Tiquia *et al.,* 1996). This study assessed the impact of moisture on the microbial concentrations of the bioaerosols. It indicated an increase in the concentration of bioaerosols from week one to week two and then a decline until week four for compost with 40% and 60% moisture content. Results indicated higher bioaerosol generation for 40% and 60% moisture contents during week two when humidity levels were between 35% - 48%. However, higher humid environments limited the bioaerosol generation and so a reduced concentration was observed with 80% moisture content. These results suggest that certain ranges of humidity would have little or no impact on the dispersal mechanism. Further, the results of the temperature and moisture dependent experiments showed an increase in the concentrations of bioaerosols at 33°C and 60% moisture content as compared to others temperatures and moisture content.

Conclusions:

The findings achieved in this research indicated maximum and minimum concentrations of bioaerosols onsite and upwind of the composting facility respectively. A difference between the microbial diversity of the compost as well as between the communities' composition of bioaerosols from upwind, onsite and downwind locations revealed that microbial populations of bioaerosols from composting sites are not absolutely traced back to the source i.e. compost. Meteorological impact indicated that a positive relationship exists between microbial abundance (bacteria & *S. rectivirgula)* and temperature and wind speed which was further justified through laboratory scale experiments. However, higher temperature negatively influences the abundance of *S. rectivirgula,* whereas it positively influences the abundance of bacteria and *A. fumigatus.*

6.3 Future work

This research has contributed in the understanding of the patterns of emissions and community composition of compost associated aerosols. However, there are certain gaps that should be taken into consideration and addressed in further studies. Firstly, there is need for a standard sampling procedure of bioaerosols in order to extensively monitor emissions from waste management facilities, such as sampling equipment, locations, time interval etc. Secondly, a molecular based approach should be utilized as an analytical tool in gaining further insights into the diversity of bioaerosols and abundance of pathogenic microorganisms. Additionally, comprehensive research is required to further elucidate the impact of seasonal parameters on the emissions and composition of bioaerosols from waste management facilities. Finally, utilizing a standard sampling protocol and molecular techniques in a detailed study, it is also essential to re-evaluate risk assessment regarding composting sites.

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APPENDIX

Statistical data supporting Chapter 3

Table 3.2 Correlation matrix of abundance of total bacteria and *S.rectivirgula* and meteorological variables for summer season

	Total bacteria	Humidity	Temperature	Wind speed	*S.rectivirgula*
Total bacteria	1				
Humidity	-0.23	1			
Temperature	0.08	-0.99	1		
Wind speed	0.52	-0.93	0.87	1	
S.rectivirgula	0.97	-0.46	0.32	0.71	1

Table 3.3 Correlation matrix of abundance of total bacteria and *S. rectivirgula* and meteorological variables for autumn season

	Total bacteria	Humidity	Temperature	Wind speed	*S.rectivirgula*
Total bacteria	1				
Humidity	0.53	1			
Temperature	-0.59	-1.00	1		
Wind speed	0.26	0.72	-0.68	1	
S.rectivirgula	0.91	0.39	-0.42	0.36	1

Table 3.4 Correlation matrix of abundance of total bacteria and *S. rectivirgula* and meteorological variables for winter season

	Total bacteria	Humidity	Temperature	Wind speed	*S.rectivirgula*
Total bacteria	1				
Humidity	-0.63	1			
Temperature	0.59	-0.81	1		
Wind speed	0.71	-0.42	0.01	1	
S.rectivirgula	0.82	-0.92	0.83	0.45	1

Statistical data supporting Chapter 4

Table 4.2 Correlation matrix of abundance of bioaerosols and meteorological variables for Waste buster composting facility, Lahore Pakistan

	Total bacteria	Humidity	Temperature	Wind speed	*S. rectivirgula*
Total bacteria	1				
Humidity	-0.50	1			
Temperature	0.18	-0.93	1		
Wind speed	-0.58	0.51	-0.28	1	
S. rectivirgula	0.71	-0.86	0.73	-0.27	1

Table 4.3 Correlation matrix of abundance of bioaerosols and meteorological variables for Lahore compost facility, Lahore Pakistan

	Total bacteria	Humidity	Temperature	Wind speed	*S. rectivirgula*
Total bacteria	1				
Humidity	-0.22	1			
Temperature	0.42	-0.97	1		
Wind speed	-0.5	0.95	-0.99	1	
S. rectivirgula	0.66	0.56	-0.38	0.30	1

Statistical data supporting Chapter 5

Table 5.2 Correlation matrix of abundance of total bacteria, *S. rectivirgula* and *A. fumigatus* (copies/m^3) from compost box at 33°C

	Total bacteria	Humidity	*S. rectivirgula*	*A. fumigatus*
Total bacteria	1			
Humidity	-0.06	1		
S. rectivirgula	0.73	-0.43	1	
A. fumigatus	0.67	-0.59	0.98	1

Table 5.3 Correlation matrix of abundance of total bacteria, *S. rectivirgula* and *A. fumigatus* (copies/m^3) from compost box at 22°C

	Total bacteria	Humidity	*S. rectivirgula*	*A. fumigatus*
Total bacteria	1			
Humidity	-0.30	1		
S. rectivirgula	0.99	-0.27	1	
A. fumigatus	0.99	-0.31	0.99	1

Table 5.4 Correlation matrix of abundance of total bacteria, *S. rectivirgula* and *A. fumigatus* (copies/m^3) from compost box at 12°C.

	Total bacteria	Humidity	*S. rectivirgula*	*A. fumigatus*
Total bacteria	1			
Humidity	-0.92	1		
S. rectivirgula	0.16	-0.45	1	
A. fumigatus	-0.20	-0.19	0.70	1

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Table 5.5 Correlation matrix of abundance of total bacteria, *S. rectivirgula* and *A. fumigatus* from compost with 40% moisture content

	Total bacteria	Humidity	*S. rectivirgula*	*A. fumigatus*
Total bacteria	1			
Humidity	-0.85	1		
S. rectivirgula	-0.06	-0.35	1	
A. fumigatus	-0.19	-0.28	0.97	1

Table 5.6 Correlation matrix of abundance of total bacteria, *S. rectivirgula* and *A. fumigatus* from compost with 60% moisture content

	Total bacteria	Humidity	*S. rectivirgula*	*A. fumigatus*
Total bacteria	1			
Humidity	-0.17	1		
S. rectivirgula	0.94	-0.14	1	
A. fumigatus	0.87	-0.07	0.98	1

Table 5.7 Correlation matrix of abundance of total bacteria, *S. rectivirgula* and *A. fumigatus* from compost with 80% moisture content

	Total bacteria	Humidity	*S. rectivirgula*	*A. fumigatus*
Total bacteria	1			
Humidity	-0.94	1		
S. rectivirgula	0.98	-0.99	1	
A. fumigatus	0.99	-0.95	0.98	1